THE BRITISH VETERINARY JOURNAL

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Vol. 111 **January, 1955** **No. 1**

EDITORIAL

A PLEA TO CLINICIANS

DURING recent years there has been a remarkable increase in the amount of material which has reached the Editorial desk of most professional journals. The urge to take up the pen is infectious but the contagion would appear to be limited in its effects to certain particular and specialised groups of workers who, at the best, represent only a small percentage of the active workers in any profession. The great majority of the members remain mute and in the Veterinary and Medical professions these are represented by the clinicians.

What is the reason for this state of affairs? A clinician's life is full of variety. In the course of a day's work there are bound to be occasions when his diagnostic and therapeutic skill is tested to the utmost. No two cases are exactly the same. A practitioner has to emulate the deductive prowess of a Sherlock Holmes to achieve a logical diagnosis, exhibit the wisdom of a Solomon in order to design a line of treatment and vouchsafe the attributes of an Oracle to prognosticate on the future. The research worker is not subjected to these kaleidoscopic demands. As a rule he works according to a preconceived and ordered plan on some specific topic: much of his work is of a routine character and calls for the exercise of a technical agility rather than for mental strain. A great deal of his work can be relegated to the attention of a competent laboratory assistant and to a typist. At the end of an appointed task the protocols can be assembled and studied in the peace of his office free from the anxieties which

(*The December issue of The British Veterinary Journal was published on December* 20)

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are associated with an importunate client and the life or death of a patient. If the work has been effected with painstaking and accurate diligence and the requisite charts and tables provided, the final evaluation of the work could be assessed easily by a person well versed in the intricacies of statistical methods. The major proportion of the MSS. which fills the columns of our journal emanates from the research institutes and workers, and in some ways it is a sign of the times.

There has been a great increase in the number of research institutions affecting all disciplines and spheres in the world of scientific endeavour. Young men or women can now enter upon a career in research safe in the knowledge that their future is secure and if fortune favours they may achieve renown and commensurable financial reward. About 20,000 so-called original articles storm the columns of the scientific press each week. Some of these articles add to the store of knowledge, some serve an immediate practical purpose, some are worth perusing for their entertainment value but many fall short of any of these virtues. One thing is common to all, be they original or pseudo-original. They provide work for others who may lack vision or ideas. The latter can occupy their time by repeating the work with the object of proving, condoning or rejecting the initial authors' contentions and conclusions. This estimable task can justify many months of uninspired effort.

Many people like to see their words in print. Letters to the editors of the popular Press are a token of this desire. It is a matter of some regret that the measure of a scientific worker's worth, and his chances of promotion, seem to be related in exact proportion to the volume of his literary output. Verbosity often carries good dividends: the modest savant who will talk only when he has something of value to disclose is at a disadvantage. Clinicians do not labour under these repressions; their reputation is measured by hard practical and economic standards. They do not need to have recourse to the Press to gain renown. None the less their clinical records, be they of failure or success, can be of great value to others and add to the store of knowledge. Such records serve as a guide to research workers and help to direct their efforts in those directions where their special knowledge and skill can be put to profitable and often immediate use. To gain full value from research the workers in the field and in the laboratory must come much closer together. Each must be cognisant of the problems which beset the other. They have much in common and the greatest factor is that they both follow the experimental approach for the solution of their problems.

Veterinary clinicians owe much to the research workers and we would urge them to repay that debt by recording their observations in the field and thereby enrich our literature as did Percival, Blain, and Youatt in the past.

GENERAL ARTICLES

EARLY PREGNANCY TESTS FROM STUDIES OF BOVINE CERVICAL MUCUS

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Introduction

SINCE it is known that blood or urine hormone tests for pregnancy, so effective for many species, are not applicable to cattle, other tests of various kinds have been proposed. These have been summarised by Cowie (1951). Since Cowie's review was published, further claims for early pregnancy tests have been made, including a number associated with observable changes in the mode of crystallisation of NaCl in the cervical mucus, or in the appearance of such mucus when fixed with $AgNO_3$.

The most encouraging research in this field is probably that described by Higaki and Awai (1953) but, even if the claims of these authors should be fully substantiated, the interpretation of such phenomena requires considerable experience, involves a strongly subjective element and is, as yet, incapable of being expressed in numerical terms.

Glover (1953) has described preliminary experiments suggesting that measurements of flow-birefringence may make it possible to distinguish pregnant from non-pregnant secretions, but this method has not yet been developed, nor is it easy to foresee how it could be adapted to practical requirements.

Scott Blair, Cowie and Coppen (1942) and Scott Blair, Cowie and Folley (1942) showed that the flow properties of bovine cervical secretions, taken from around the cervical os, change during pregnancy, but more than ten years elapsed before Glover and Scott Blair (1953) were able to suggest a technique by which pregnancy could be detected with over 90 per cent accuracy in normal cows at six weeks. The method of test and the apparatus required were rather cumbersome and each cow had to be sampled on many occasions, a process believed to affect the consistency of the secretions and to diminish the accuracy of the test. No *ad hoc* test for early pregnancy was claimed in this paper.

It is now appreciated that the critical factor is the method by which the sample is taken and the frequency of sampling. The failure to develop a method of early diagnosis twelve years ago is now ascribed largely to the lack of appreciation of the importance of the exact method of sampling.

Secretions are now taken through a vaginal speculum with an endometrial curette (with the cutting edge rounded off) which is inserted 2 to 3 cm. into the cervix and twisted so as to attach the secretion to it. It is important not to get contamination from the vagina. The samples, which must be tested within an hour or so, should be stored in very small containers to avoid unnecessary drying.

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Following the publication of this article, further experiments (unpublished) in which samples were taken only once from each cow at six weeks after conception, raised the level of correct pregnancy diagnosis to 96 per cent for 50 normal cows.

It therefore seemed advisable to explore the possibilities of developing simpler tests which would involve taking (normally) only one sample from the cow.

It was at one time thought that the wide individual fluctuations in the readings shown in the figures in the 1953 article corresponded to spontaneous changes in hormonal levels in the animal and precluded the use of single tests for diagnosis. It now appears, however, that, although this is occasionally so, e.g., when pregnant animals come into œstrus, the main cause of the fluctuations was almost certainly connected with the frequent sampling of the animals. It seems that a very simple test of over-all consistency will indicate pregnancy at an early date in most cases, if the correct sampling technique is adopted and if one sample only is taken from the cow.

The reason for the connection between flow properties and hormonal levels is still obscure and it therefore seemed advisable to investigate other physical methods. Glover (loc. cit. 1953) published preliminary data suggesting that the ultra-violet absorption spectra of aqueous dispersions of cervical secretions (under standardised conditions) change progressively from œstrus, through diœstrus into pregnancy, but the method was too cumbersome to be of much practical value.

Before proceeding to simplify either test, it was necessary to inquire whether a test for pregnancy at four or five weeks would be of any value. Some veterinarians claim a high degree of diagnostic success using rectal palpation at 40 days or even earlier. Unfortunately, no data were available to test such claims by statistical methods but our limited experience, together with advice from a number of veterinary surgeons, suggests that the skill necessary to reach a 90 per cent level of correct diagnosis at less than six weeks is exceptional and any test which improves on this is of practical value.

The purpose of the present paper is to describe a flow test which has been used on some 130 cows in a single herd, and a spectroscopic technique which, while lending itself to considerable further simplification, shows promise of providing an alternative and independent method.

A simple method for measuring over-all consistency of mucus. In our earlier paper (loc. cit. 1953) a "Rheoscope" was described in which a small quantity (0.006 ml.) of cervical secretion is extruded from a glass capillary tube (0.07 cm. diam.) at a constant rate (1 cm./min.), the varying pressure needed to maintain this rate being plotted on a chart against the diminishing length of the column. The slope of the curves is negative at œstrus (i.e., the pressure must actually be increased to maintain a constant rate of extrusion), small in diœstrus and large in pregnancy. This complicated procedure involving steady flow has many advantages for research purposes; but a new and simplified instru-

ment has now been made, for use mainly with pregnancy diagnosis. It operates at a constant pressure and an over-all (average) rate of extrusion is measured. A modification in the way the instrument is used also enables flow-elasticity to be measured which is, in general, diagnostic of the onset of œstrus (see Scott Blair, Folley, Malpress and Coppen (1941) who describe a simple device " the œstroscope," for detecting œstrus in cows). To facilitate discussion, some name is required for the new and simplified apparatus and this will be referred to as the " Consistometer."

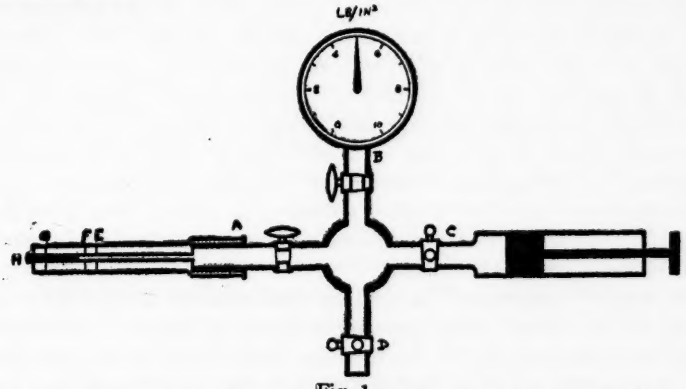

Fig. 1
Apparatus for measurement of over-all consistency of cervical mucus (" Consistometer ").

The consistometer (Fig. 1) consists of a small air-reservoir connected to four valves. These are attached to a capillary tube (A) exactly similar to that used on the rheoscope, a pressure gauge (B), a small pump working on the principle of a hypodermic syringe (C) and a release to the outside atmosphere (D). The capillaries are graduated with three marks (E, F and G in the figure). Closing valves B and D, the secretion, held on the point of a pin, is sucked into the capillary to approximately the mark E, forming a column 16 mm. long, the excess then being removed from the end with the finger. A is then closed, B opened and a convenient pressure, as recorded on B, is built up within the reservoir, which is large compared with the very small volume of secretion. The valve C is then closed and A opened. The secretion starts to flow out of the tube, forming a blob (H) at the end. When the meniscus passes the mark F, 3 mm. from E, a stop-watch is started, and the flow is timed over 1 cm. to G, a point 3 mm. from the open end of the tube. Since the pressure of extrusion is chosen from the appearance of the secretion and its behaviour in filling the capillary, it is as well to repeat the test, often at a different pressure, preferably on another sub-sample of mucus, of which there is usually enough for several tests. About 0.1 ml. of sample is generally available. This should be mixed gently with a small spatula before dividing into sub-samples, the part not in use being put back in the container so as to minimise loss of moisture. Since it is found best to use outflow times $\sim$ 10 sec., several tests can be completed in about ten minutes, the capillary being rinsed out with water between the

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tests, without disconnecting it from the apparatus. The inside of the capillary should be moist: a very small drop of water behind the secretion provides a good meniscus and should not dilute the sample appreciably. After a few tests the tube should be changed and cleaned thoroughly in hypochlorite solution. In view of the enormous range of consistency of the samples, a fairly wide scatter between replicates is allowable.

If cervical secretions were simple fluids, the time of outflow (t) would be inversely proportional to the pressure (P) and the product Pt, multiplied by a constant derived from the dimensions of the capillary, would give the viscosity. In fact, secretions behave in a much more complex manner, but it is found that, for values of t from about 5 to 60 sec., the product $Pt^{\frac{1}{2}}$ is approximately independent of pressure, i.e., a scattergram plotting log P against log t, for a number of tests on a single secretion, has a slope of about $\frac{1}{2}$. The average value for (normally) from 2 to 5 runs on a secretion is taken as a measure of its "consistency." Fortunately, "consistency" in this sense does not vary much with temperature as does a true viscosity; it varies over a wide range ($\sim$ 10,000 : 1) with the physiological condition of the cow, so that no temperature control for the apparatus is needed.

The term "consistency" is used by rheologists to mean "That property of a material by which it resists permanent change of shape . . . defined by the complete flow-force relation." Such terms, which do not have a precise dimensional connotation, are often useful and it is the resistance to flow under an arbitrarily selected set of conditions which is here expressed as "consistency."

The "consistency," so defined, would reduce to a viscosity in the case of a true fluid. For mucus, it must be regarded as a kind of average of a series of viscosities under an arbitrary but reproducible set of changing conditions. (Those interested in the physics may consult the chapter on "Secretions" by one of us [G.W.S.B.] in a book edited by Frey-Wyssling [1952]).

Pathological conditions, especially within the cervix, may change the consistency of the secretions drastically. All pregnancy tests which depend on properties of cervical mucus are *not applicable to such cases* and the data from cows in which relevant pathological symptoms were observed are not included in these records.

With this exception, all secretions too thick to be drawn into the capillary were classified as from "pregnant" animals and all those which were so thin that no adequate test could be performed on them were classed as from "non-pregnant" animals. The latter group would include all œstrous samples and can be tested for flow-elasticity as follows:—

The capillary is filled to E and the excess at the end removed. B and D are kept closed and the secretion is gently extruded by exerting a steady pressure from C, until the blob H just begins to show signs of falling away from the mouth of the capillary. At this moment the valve D is opened, thereby releasing the pressure instantly. Oestrous samples flow back for most of the way along the tube. ("Flow-elasticity.") This serves as a test for œstrus in those cases (rare in Britain) when there is not enough secretion for the "œstroscope" to be used.

The hypothesis to be tested is that a value of $Pt^{\frac{1}{2}}$=C can be chosen such that secretions having a higher consistency than C occur in pregnancy in normal cows, whereas if $Pt^{\frac{1}{2}}$ is less than C, the cow is not pregnant. The value of C is selected so as to equalise, as nearly as possible, the number of wrong diagnoses of pregnant and of non-pregnant cows. A provisional value of C=9.0 lb./in.^{2}sec.$^{\frac{1}{2}}$ (=630 g/cm.^{2}sec.$^{\frac{1}{2}}$), chosen early in the experiment, has not been changed. In a similar way, an angle of $\theta = 57$ deg. ($\tan \theta = 1.54$) is selected as critical for the rheoscope. These values may be improved in the light of future experience.

In most cases, samples were large enough to be tested on both the rheoscope and the consistometer. During the greater part of the experiment, it was not appreciated that the consistency of the secretion may be changed because of a previous sampling, and some cows were sampled several times. This probably reduced our successes and accentuated the problem of the circumstances whereby more than one test on the same cow should be included in the results.

Selection of Data for Determining Efficiency of Pregnancy Diagnosis. The following rules were drawn up:—Data from cows with relevant pathological symptoms and those from cows in such condition as the first week of pregnancy or immediately after parturition, are excluded. Data from cows known to be subfertile (defined as having required more than three inseminations) are included if there is no observed pathological cause for the infertility; but, since it is already known that the test sometimes fails in such cases, they are specially indicated as "subfertile." Successful diagnoses of subfertile cows are, of course, also likewise indicated.

Data from the repeated tests on the same cow are included only when a cow found normal before service was tested also in pregnancy or in two pregnancies within the thirteen months of the experiment. Tests on cows which had been found to be abnormal in an earlier pregnancy are only included when the earlier abnormality was found before the start of the present experiment. This is because it is known that abnormal conditions of secretions are apt to persist and recur in certain cows. There was a tendency to test abnormal cows a number of times and such tests should not count as independent failures, since normally, cows were only tested once.

When a non-pregnant cow gives a correct test but, on repeating, gives an incorrect test, both are counted, since the second test would have been expected to be trustworthy; whereas a failure, followed by a second failure on the same cow would count as a single failure. If a cow is tested twice, once successfully and once wrongly, both tests are included.

An exception is made in the case of samples at four weeks of pregnancy, since failures as early as this are not necessarily believed to imply abnormality. Many cows were tested at both four and five weeks and, in such cases, both tests are included. If a later test failed, all three tests are included, but the undue preponderance of the abnormal cow is balanced by repeat tests at the same stages at pregnancy from a normal cow. (Only two such cases occurred.)

In a very few cases the average reading falls very close to the criterion

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value and there is considerable scatter amongst the replicates. Such tests were discarded and repeated as soon as possible.

Results of diagnoses using the Rheoscope and the Consistometer.

It is not our purpose to discuss the use of the rheoscope for early pregnancy diagnosis, except in so far as the data help to validate or invalidate the use of the much simpler consistometer. In most cases, the two instruments give the same diagnosis, be it right or wrong. The correlation coefficient between the slope of the rheoscope curve (as a tangent) and the mean value of $Pt^{\frac{1}{3}}$ on the consistometer for 141 samples is .71. The true figure must be considerably higher than this, since it was necessary to omit all samples which were too thick or too thin to be tested adequately on the rheoscope, which does not have as wide a range as the consistometer.

With the exception of those cows with disease, there were almost exactly the same number of cases in which the rheoscope was correct and the consistometer mistaken as the reverse. There was a tendency for there to be more errors with the rheoscope in non-pregnant cows and the consistometer tended to fail more often in pregnancy. This is, of course, related to the arbitrary selection of θ and C, suggesting that the former should perhaps be raised and the latter diminished slightly.

Subject to the conditions quoted above. Table I shows the efficiency of the consistometer as a method of detecting early pregnancy. The table refers to 126 cows from the N.I.R.D. herd, some Shorthorn, some Friesian and some Guernsey. Duplicate tests, not included in the table, give support to the conclusions.

During the course of the experiment (13 months) about 240 secretions were tested on the consistometer.

TABLE I

The results of pregnancy diagnosis using the "Consistometer."

	Non-Pregnant	Pregnant				
		4 weeks	5 weeks			6 and more weeks
			Previously tested	Not previously tested	Total	
No. of correct diagnoses	49	26	25	21	46	16
No. of incorrect diagnoses	5	8	6	1	7	2
% correct	91	77	81	96	87	89

Efficiency of Consistometer diagnosis of bovine pregnancy.

The true efficiency of the tests is higher than the data in Table I would suggest, for the following reasons:—

Two of the five mistakes on non-pregnant cows occurred in cases of known sub-fertility. (Four subfertile cows were correctly diagnosed as non-pregnant.) Two other cows were, at 7 weeks, stated to be not pregnant by the veterinarian but had given positive diagnoses on the consistometer at four weeks. Although

here listed as errors, such cases may be due to early fœtal death (see Stewart, 1952). The one remaining wrong diagnosis is unaccountable.

Wrong diagnoses were made on two pregnant cows well into pregnancy; these errors are listed at each stage and they account for one of the eight failures at four weeks, two of the seven failures at five weeks and both the failures at six weeks. No pathological signs were observed, though in one case, the secretion was " unhealthy " at four and five weeks and, in the other case, the temperature of the cow at four weeks was 104.2° F. Repeated sampling may account for the continued failure of the test in such rare cases.

Of the 53 cows tested at five weeks, some had also been tested at four weeks. Though the numbers are small, the data in Table I suggest that when a secretion giving a " pregnant " diagnosis is removed from the cervix at four weeks, a sample taken a week later may give a figure falling below the critical level, even though the cow is pregnant.

Of about 130 cows tested during the course of the year, seven suffered from disease conditions liable to invalidate the test. These were: ovarian cyst, cervical erosion, hydrosalpinx, retained corpus luteum and degenerated ovary.

Ultra-violet Absorption Spectra. An improved method for obtaining the ultra-violet spectra of secretions has been developed by direct measurement of the absorption of undiluted samples, using a Unicam Spectrophotometer. Dicestrous and pregnancy samples are spread between two fused quartz plates, forming a cell of thickness 0.1 mm. Estrous samples are examined in standard 2 mm. spectrophotometer cells. The preparation of the cell and plotting of the spectrum between 200-400 mμ can be completed in half an hour for each sample.

No qualitative differences appear among the various types of secretion, which are completely opaque for wavelengths below 200 mμ and perfectly transparent in the visible range. At intermediate wavelengths they all tend to show a maximum at about 278 mμ and a minimum at about 255 mμ.

It is found, however, that at the maximum, 278 mμ, the absorption (per cm. length) for pregnancy samples is greater than that for dicestrous samples which, in turn, is greater than that for cestrous samples. Table II gives a summary of these preliminary results on 45 cows. ΔD is the absorption where the maximum occurs at 278 mμ minus the general absorption at the same wavelength, determined by interpolating the rest of the smooth curve. The thickness of the cell is d cm. The concentration of specific chemical components of the mucus has not been taken into account since the first aim has been to investigate the possibilities of the method as a quick test for pregnancy.

TABLE II

Ultra-violet Spectra of Secretions from 45 cows.

Physiological condition	Number of samples	Average $\Delta D/d$ (cm.$^{-1}$)	Standard Deviation
Pregnant	23	5.3	2.3
Dicestrous	15	2.1	1.8
Œstrous	7	0.2	0.08

Density of absorption band at 278 mμ in mucus from cows in pregnancy, dicestrus and œstrus.

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The data in Table II suggest the possibility of the use of the spectrophotometer as an alternative test for early pregnancy. It is hoped to study further the fundamental processes underlying these findings.

Discussion

It has been suggested that any technique involving the collection of samples of mucus from the cervix of the cow may involve a risk of introducing infection. We have no evidence of this. Over a period of four years about 250 pregnant cows have been sampled, some as often as once a week throughout the complete gestation period, and some 1,500 samples of mucus have been taken. Freshly sterilised instruments were used, there was no evidence that the use of the speculum in the vagina led to an increased incidence of *Vibrio fœtus*, and the incidence of abortion was no higher than normal.

From other work, both published (Glover and Scott Blair, 1953) and unpublished, it is known that the consistency of secretions is affected greatly by ovarian hormonal activity, but it is not possible to diagnose, from the consistency, such conditions as ovarian cysts and retained corpora lutea, the presence of which is liable to invalidate the pregnancy test.

One of the major advantages of the consistometer test is that it can be used some weeks before a diagnosis of pregnancy could be made by a clinician. The consistometer test cannot help in comparatively rare disease conditions and under these circumstances diagnosis by rectal palpation must be made at a later date.

In practice, the sampling and testing of the mucus in the consistometer takes no more time than an examination by rectal palpation. Extra time is required for sterilising the instruments and the only additional equipment needed is the consistometer.

The aim of the present work is to develop an objective method of pregnancy diagnosis which will compare favourably in reliability with the highly subjective method of rectal palpation. Palpation demands a skill which needs considerable training to acquire and continual practice to maintain. Moreover, after examining a few animals only, the operator fatigues and his skill declines. The methods described in this paper give results virtually independent of the operator. Some 30 per cent of the consistometer tests were done by a laboratory assistant after about half an hour's instruction and were as satisfactory as those done by the skilled operator.

An American writer has recently expressed the advantage of objective tests: "When description gives way to measurement, calculation replaces debate."

Summary

A rapid and simple method is described for measuring an arbitrary "over-all consistency" of samples of mucus taken in a prescribed way from the bovine cervix. Excluding animals showing well-defined disease processes of the genital system (5 per cent of the population examined) it is found that in

pregnancy the "consistency" generally exceeds a certain value and that in non-pregnancy it is less than this critical value.

In this way, pregnancy has been diagnosed correctly in 77 per cent of cases four weeks after conception and in 87 per cent of cases after five weeks. The latter figure is increased to 96 per cent if cows which have been previously sampled are excluded.

Non-pregnancy was determined correctly in 91 per cent of cases, the errors being generally associated with sub-fertility or suspected early fœtal death.

Preliminary experiments suggest that the intensity of a band in the ultra-violet absorption spectra of cervical secretions is smallest at œstrus, somewhat larger at dioestrus and greatest in pregnancy. This may lead to a comparatively simple alternative test.

Acknowledgment

We wish to acknowledge the help of Mr. D. R. Shorter, M.R.C.V.S., who made the pregnancy diagnoses by rectal palpation and determined the pathological conditions; and also of Mr. E. J. Pinnegar, of the Engineering Department, N.I.R.D., who made for us the consistometer described in this paper.

REFERENCES

Cowie, A. T. (1951): *Vet. Rec., 63,* 371.
Frey-Wyssling, A. (1952): "Deformation and Flow in Biological Systems." Amsterdam: North Holland Publ. Co.
Glover, F. A., and Scott Blair, G. W. (1953): *J. Endocrin., 9,* 160.
Glover, F. A. (1953): *Nature* (Lond.), *172,* 255.
Higaki, S., and Awai, Y. (1953): *Bull. Nat. Inst. Agric. Scis.* (Chiba, Japan), Series G. (No. 7), 59, 66, 76.
Scott Blair, G. W., Folley, S. J., Malpress, F. H., and Coppen, F. M. V. (1941): *Vet. Rec.,* November 29.
Scott Blair, G. W., Cowie, A. T., and Coppen, F. M. V. (1942): *Nature* (Lond.), *149,* 609.
Scott Blair, G. W., Cowie, A. T., and Folley, S. J. (1942): *J. Physiol., 101,* 11P.
Stewart, D. L. (1952): *Vet. Rec., 64,* 303.

THE EGGS OF *HYOSTRONGYLUS RUBIDUS* HALL, 1921. A STOMACH WORM OF THE PIG, AND THEIR RECOGNITION IN PIG FÆCES

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It must be rare for an error in an original description of a parasite to be perpetuated for so long and by so many writers as has occurred with the size of the eggs of *Hyostrongylus rubidus.*

In 1952 we decided to see what eggs occurred in pig fæces, since routine dosing with sodium fluoride has reduced the incidence of *Ascaris lumbricoides.* Two helminths in particular were looked for, *Œsophagostomum dentatum* and *Hyostrongylus rubidus,* both of which occur in Great Britain. Information was sought as to the size and other features of the eggs of these two species.

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A textbook of veterinary helminthology and entomology (Mönnig, 1950) states that the eggs of *H. rubidus* measure 45 x 36 μ, yet the egg of this species illustrated in a plate at the front of the book measures about 70 x 35 μ. The illustrations for this composite plate of the eggs found in the fæces of domestic animals were provided by a number of contributors. A recently published booklet for veterinary practitioners (B.V.A., 1952) gives the size as 45 x 35 μ, and provides an air-brush drawing of an egg of this size (Fig. 22) with the statement that the eggs must be distinguished from those of *O. dentatum* which are similar but larger. Other texts which give the same egg size include those of Foster (1912), Baylis (1929), Neveu-Lemaire (1936) and Cameron (1951). In the textbook of Sprehn (1932) the size is given as 45 x 38 μ. In contrast, one British veterinary textbook (Boddie, 1950) states correctly that the eggs measure 70 x 36 μ and can be recognised in the fæces only tentatively.

Goodey (1924) in his excellent description of *H. rubidus* and its larval development does not mention the size of the eggs directly, but his line drawing suggests they are about 70 x 40 μ.

Others, such as Alicata (1935), who have actually studied the worm and its larval stages, are quite clear that the eggs are about the same size as those of *Œsophagostomum* spp. and of many other gastro-intestinal helminths of livestock. Alicata (1935) gives the size as 60 to 76 x 31 to 38 μ and states that in fresh fæces the eggs are usually in the early tadpole stage of development, whereas those of *O. dentatum* are usually in the 8 to 16 cell stage. When the fæces are some hours old the more rapid development of *O. dentatum* allows them to catch up with those of *H. rubidus* and makes differentiation difficult when eggs of both species are present together. Recent American publications, such as those of Morgan and Hawkins (1949) and of Coffin (1945) quote Alicata's measurements.

It is of interest to know how the error occurred; if the small eggs are looked for in pig fæces, the parasite will not be detected, and this may mean that a number of observers failed to recognise them. The adult worm, too, is easily overlooked for it is very small and, embedded in mucus, is seen as fine reddish threads lying on or in the gastric mucosa.

The original article by Hassall and Stiles (1892) states (p. 209): "Eggs 0.045 x 0.036; clearage begins in the mother." The length of the egg is incorrect and a misprint in the same line occurs ("clearage" for "cleavage"). This seems to be the origin of the error, repeated with slight variations by so many writers for some 60 years, in publications from South Africa, Germany, Canada, U.S.A. and Britain, and doubtless elsewhere.

It is probable that the measurements given by Hassall and Stiles (1892) should have been 0.065 x 0.036 mm. A mistake in manuscript or proof is the likely explanation. That so many later authors have perpetuated this error means that few of them can have measured the eggs within the adult female worm or actually seen them in pig fæces. As a result, it is likely that many who have examined pig fæces will have overlooked the eggs of *H. rubidus,* as we did. It was the finding of infective larvæ of *H. rubidus* in fæces cultures that first sug-

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gested to us that the eggs were, in fact, much larger than many authors stated and led us to search for them in pig fæces.

Observations

The size of *Hyostrongylus* eggs was determined by examining female adult worms which had been preserved in formalin for months and came from three groups of pigs in the North Riding of Yorkshire.* The eggs lying within the uteri were about 68 x 36 microns. Although the eggs were distorted by fixation and not fully developed, being mostly in the one-cell stage, it was evident that they approximated to the size quoted by Alicata (1935).

Further evidence as to egg size was obtained when two experimental pigs at the laboratory were found to contain about 100 adult *Hyostrongylus* when sent to the bacon factory. The adult worms were just discernible as fine reddish threads entangled in thick gastric mucus. They were confined to the lesser curvature and were mostly in groups. The very small males (4 to 5 mm.) were particularly difficult to find. Eggs expressed from females by gentle pressure measured 70 to 76 x 36 to 39 microns and were usually at either the 32-cell or " tadpole " (indented) stage,, as mentioned by Alicata (1935). When the eggs were cultured for six days in sterilised pig fæces they yielded the characteristic third stage *Hyostrongylus* larvæ.

Having found adult *Hyostrongylus* in the laboratory pigs it was decided to see how common the eggs of this worm are in pig fæces. During the last two years we have examined microscopically several hundred samples of pig fæces, primarily for lungworm eggs. In addition, fæces cultures were made from 53 pigs on 14 farms in different parts of the country. Cultures from 3 pigs were negative for sheathed third stage larvæ, those from 20 yield larvæ of *Œsophagostomum dentatum,* 3 gave *Hyostrongylus rubidus* only, and the remaining 27 gave larvæ of both species. *Hyostrongylus* larvæ were thus isolated from 30 of 53 samples, representing 10 of the 14 farms. *Œsophagostomum* larvæ usually greatly outnumbered *Hyostrongylus* larvæ in mixed cultures and usually accounted for 90 to 95 per cent of the total larval population. The number of larvæ from 2 gramme samples of fæces cultured at 26° C. for seven days ranged from one single specimen to several thousand. Larval counts amounted to 20 to 80 per cent of the egg counts as determined by the McMaster method or by flotation with saturated sodium chloride or magnesium sulphate. Most of the samples came from sows or large bacon pigs.

Hyostrongylus and *Œsophagostomum* were the only two types of sheathed third stage larvæ encountered. *Hyostrongylus* was distinguished by its more active movement which took it up and down in the fluid as well as horizontally. *Œsophagostomum* larvæ were more sluggish. *Hyostrongylus* larvæ are also longer and thinner than *Œsophagostomum* larvæ and when killed by heat they tended to lie straight, whereas *Œsophagostomum* larvæ usually lay curved and showed a marked wrinkling of their sheaths. Measurements of the larvæ agreed closely with those given by Goodey (1924) and Alicata (1935).

* Supplied by Mr. K. G. Towers, B.V.Sc., M.R.C.V.S.

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Measurements of eggs from fresh female *Œsophagostomum* showed that they were slightly shorter and wider (70 x 40 microns) than those of *Hyostrongylus*, as pointed out by Alicata (1935), and were less segmented (4 to 32 cells). These differences are not sufficient, however, to allow the eggs of the two species to be differentiated microscopically in pig fæces. We made numerous measurements of one or both species of egg, where identity was confirmed by fæces culture, and were unable to distinguish the two types of egg with certainty. Fæces culture is necessary for differentiation and in our hands it proved both simple and reliable.

Opinions vary as to the pathogenic significance of *Hyostrongylus*. In this country Blackwell (1923) attributed the death of a pedigree large black sow to a heavy infestation, and Blissett and Little (1930) considered it to be concerned in an obscure illness in pigs at the National Institute for Research in Dairying at Shinfield. Cameron (1951), in his textbook, states that *Hyostrongylus* is very common in England but has not been recorded in Scotland: he seems doubtful as to whether it causes the gastritis which is often associated with its presence. Castle (1932) mentioned a verminous gastritis of sows in the eastern counties of England, with wasting, inco-ordination and weakness. Lactation was suggested as a factor predisposing to clinical symptoms.

In Australia (Clay, 1938; Roberts, 1940) and New Zealand (Marshall, 1934) infestations have been stated to cause deaths. In the United States, especially in the southern states, infestations are common and treatment with carbon bisulphide (Bozicevich and Wright, 1930) or control by sanitation (Porter, 1939) has beeen recommended. Porter (1940) found, however, that experimentally infected pigs gained as much weight as control animals and showed no clinical symptoms.

In Hungary infections are said to cause serious interference with pig breeding (Kotlán and Vajda, 1939).

The parasite is thus widespread throughout the world and has often been alleged to caused illness and even deaths. The usual lesion is said to be a diphtheritic gastritis with clusters of worms embedded in mucus in the ulcerated areas.

Our own experience of fæces cultures confirms that the parasite is common in pigs but egg counts have been low, usually well below the levels for *Œsophagostomum dentatum*.

Discussion

The mistaken references in so many texts to the eggs of *Hyostrongylus* as only about half their actual size, along with the difficulty in recognising the adult worms unless the mucosa of the freshly opened stomach is examined carefully (preferably with the help of a hand lens), may account for the paucity of references to the worm in this country. It may be that serious illness can result from heavy infestations if accompanied by poor nutrition and management in young pigs or the strain of lactation in sows. The typical third stage infective

larvæ are easily recognised in fæces culture but the eggs in fæces cannot be distinguished with certainty from the eggs of *Œsophagostomum dentatum.*

Summary

Since the original description of *Hyostrongylus rubidus* by Hassall and Stiles in 1892 many authors have continued to quote the size of the eggs incorrectly as 45 x 36 microns. The eggs measure about 70 x 35 μ and cannot be distinguished microscopically in pig fæces from those of *Œsophagostomum dentatum.* The two species are easily distinguished in fæces culture by their characteristic third stage infective larvæ. *Hyostrongylus* larvæ were found in fæces culture in 30 of 53 samples of pig fæces from 10 of 14 farms in different parts of the country. The error in size, which is apparently a misprint, may have led many observers to fail to find the eggs in pig fæces.

REFERENCES

Alicata, J. E. (1935): "Early Development Stages of Nematodes Occurring in Swine." *U.S.D.A., Tech. Bull.,* 489.
Baylis, H. A. (1929): "A Manual of Helminthology, Medical and Veterinary. London: Baillière, Tindall and Cox.
Blackwell, W. G. (1923): *Vet. Rec., 3,* 279.
Blissett, A. H., and Little, W. L. (1930): *Ann. appl. Biol., 17,* 171.
Boddie G. F. (1950): "Diagnostic Methods in Veterinary Medicine," 3rd Ed. Edinburgh: Oliver and Boyd.
Bozicevich, J., and Wright, H. D. (1935): *Vet. Med., 30,* 390.
British Veterinary Association (1952). Laboratory Aids to Diagnosis. Publication No. 21. London: B.V.A.
Cameron, T. W. M. (1951): "The Parasites of Domestic Animals." 2nd Ed. London: A. and C. Black.
Castle, A. F. (1932): *Vet. J., 88,* 474.
Clay, A. L. (1938): *Austral. vet. J., 14,* 194.
Coffin, D. L. (1945): "Manual of Veterinary Clinical Pathology." New York: Comstock Publishing Company.
Foster, W. D. (1912): "The Roundworms of Domestic Swine." *U.S.D.A. Bull.,* 158.
Goodey, T. (1924): *J. Helminth., 2,* 191.
Hassall, A., and Stiles, C. W. (1892): *J. comp. Med. Vet. Arch., 13,* 207.
Kotlán, S., and Vajda, F. (1939): *Allatorv. Lapok, 62,* 233 and 255. (Abstr. in *Vet. Bull.,* 1943, *13,* 636.)
Marshall, D. (1934): *New Zealand J. Agric., 49,* 65.
Mönnig, H. O. (1950): "Veterinary Helminthology and Entomology." 3rd Ed. London: Baillière, Tindall and Cox.
Morgan, B. B., and Hawkins, P. A. (1949). "Veterinary Helminthology." Minneapolis: Burgess Publishing Company.
Neveu-Lemaire, M. (1936): "Traité d'helminthologie Médicale et Vétérinaire. Paris: Vigot Frères.
Porter,, D. A. (1939): *Proc. Helm. Soc. Wash., 6,* 21.
Porter, D. A. (1940): *Proc. Helm. Soc. Wash., 7,* 20.
Roberts, F. H. S. (1940): *Austral. Vet. J., 16,* 259.
Sprehn, C. E. W. (1932): "Lehrbuch der Helminthologie." Berlin: Gebrüder Borntträger.

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ON THE LIFE-CYCLE OF *DIROFILARIA IMMITIS* IN THE DOG AS THE FINAL HOST

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Introduction

AMONGST the parasitic nematodes of the Filariidæ are several species that are noxious to man and animals. Advances in the knowledge of the life-cycle of the Filariidæ were made by the discoveries of Manson on the intermediate host, and by Fülleborn and others on the method of invasion into the final hosts. Manson's discovery (1877, 1878, 1884) that a species of mosquito (*Culex fatigans*) was the intermediate host of *Wuchereria bancrofti,* led to the presumption that probably other filarid species also required blood-sucking arthropods as intermediate hosts. The method of invasion of the final host by infective larvæ was studied by Grassi and Noé (1900), Bancroft (1903), and Fülleborn (1907, 1908), and it was proved that when the mosquito intermediate host sucks blood the infective worm larvæ enter the final host percutaneously. It has since been found that a similar mechanism occurs with the infective larvæ of other filarid worms.

The development of the infective larvæ and the path of migration after invasion of the final host by the parasite to its ultimate location have remained unknown in all species of the Filariidæ. When the life-cycle of any one species in its final host is elucidated it will play the rôle of a key to the solution of similar problems in other species, for the habits of filarid worms resemble one another.

Since 1935 the writers have continued their studies on *Dirofilaria immitis,* and the results of these studies, particularly with reference to the life-cycle in the final host, the dog, are dealt with in this paper.

Observations on Autopsies of Naturally Infected Dogs

D. immitis infective larvæ measure only about 1 mm. long when transmitted percutaneously to the dog by the bite of a mosquito. The youngest worms found in the heart and pulmonary artery, which is their normal location, of the dog measure several centimetres. Developing parasites, intermediate between these two sizes, have never been observed. To gain information about this, autopsies of naturally infected dogs were started in August, for at this time mosquitoes breed actively in Tokyo, and the infection with *D. immitis* must then, presumably, be at its height. The autopsies were continued until February of the next year. Thorough examinations of 213 dogs were made and young worms were only detectable in the heart and pulmonary artery. The smallest

PLATE I

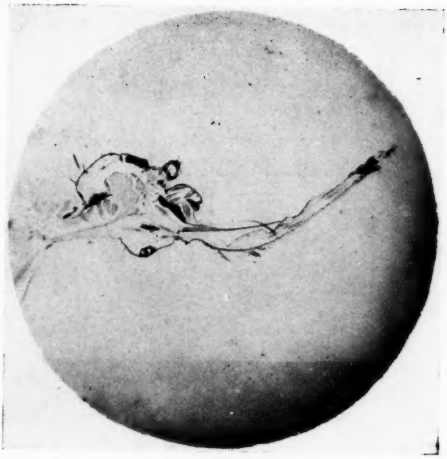

Fig. 1

Fig. 2

Fig. 3

Fig. 1
Infective *D. immitis* larvæ in the proboscis of *Aedes togoi* (longitudinal section) x 50. This mosquito was kept alive for 12 days after it had bitten a dog that became infected.

Fig. 2
Infective larva invading the body of a dog. The larva, in longitudinal section, is in the corium. The specimen was fixed in formalin solution 24 hours after the dog had been bitten by mosquitoes carrying infective larvæ in their proboscides. x 220.

Fig. 3
Three young worms recovered from the intermediate locations. They measure 6.0 cm (♂), 2.5 cm. (♂) and 1.4 cm. (♀).

(*Article by Kume, page* 16)

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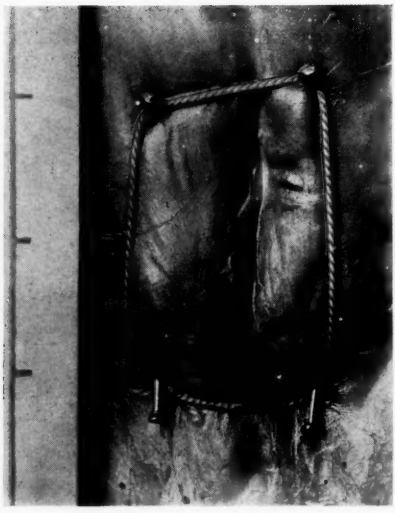

Fig. 4

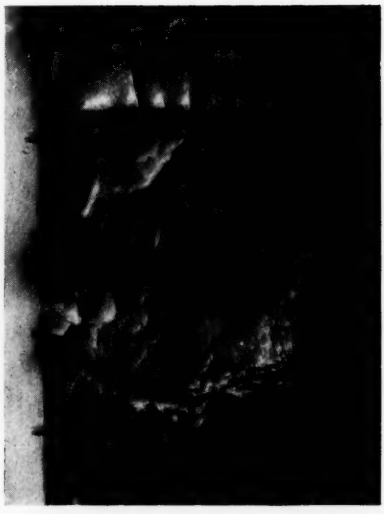

Fig. 5

Fig. 6

Fig. 4
A young worm in the submuscular membrane of the *M. scapularis.*
Fig. 5
A young worm in the subserosa of the peritoneum.
Fig. 6
Transverse section of a young worm in the submuscular membrane of the *M. gracilis.* x 200

(*Article by Kume, page* 16)

PLATE III

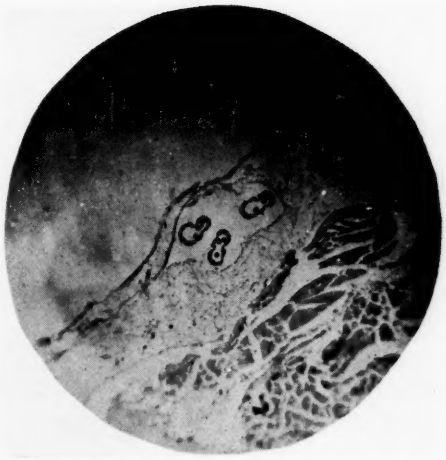

Fig. 7

Fig. 8

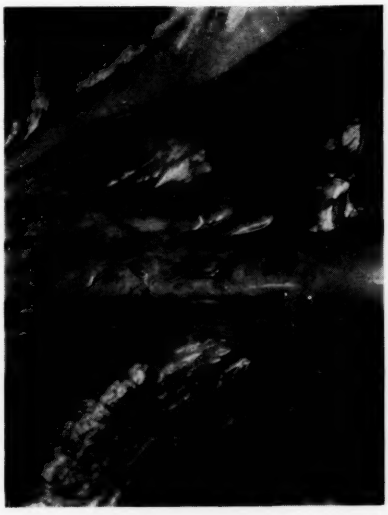

Fig. 9

Fig. 10

Fig. 7
Three transverse sections of one young worm in the adipose tissue of the fore-limb. x 56.

Fig. 8
Four transverse sections of one young worm in the *M. infraspinatus.* x 56.

Fig. 9
Young worms invading the subserosa of the vena cava caudalis.

Fig. 10
Young worms invading the subserosa of the vena cava caudalis.

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Fig. 11

Fig. 12

Fig. 11
Transverse sections of the same young worm shown in Fig. 10. x 125.

Fig. 12
Five transverse sections of one young worm attached to the intercostal vein. x 56.

(*Article by Kume, page* 16)

parasite found was 5.0 cm. long, and the majority had reached a greater length than this, as shown in Table I.

TABLE I.

Relation between parasitic locus and the developmental stage.

LENGTH OF WORM (cm)	PARASITIC LOCUS AND NUMBER OF WORMS					
	NATURAL INFECTION		ARTIFICIAL INFECTION			
	RIGHT VENTRICLE OF HEART & A. PULMONALIS		INTERMEDIATE LOCATIONS		RIGHT VENTRICAL OF HEART & A. PULMONALIS	
1.1 - 1.9			5	6		
2.0 - 2.9			8	10		
3.0 - 3.9			10	10	6	8
4.0 - 4.9			12	15	33	12
5.0 - 5.9	1	1	9	11	55	26
6.0 - 6.9	3	8	7	5	48	27
7.0 - 7.9	9	7	3		33	27
8.0 - 8.9	13	6	1		10	11
9.0 - 9.9	17	10		4	10	13
10.0 - 10.0	24	23			8	7
11.0 - 11.9	17	14		1	16	6
12.0 - 12.9	11	17			24	4
13.0 - 13.9	10	8			27	3
14.0 - 14.9	2	9			19	4
15.0 - 15.9		7			18	4
16.0 - 16.9		2			4	4
17.0 - 17.9		1			1	4
18.0 - 18.9					1	5
19.0 - 19.9		1				8
20.0 - 20.9		1				8
21.0 - 21.9						11
22.0 - 22.9						11
23.0 - 23.9						25
24.0 - 24.9						26
25.0 - 25.9						17
26.0 - 26.9						9
27.0 - 27.9						1

In this series of autopsies the first worm was found on October 16, in the pulmonary artery. Before this date none had been found in any organs or tissues of 56 animals dissected. In 58 (37 per cent) of the remaining 157 dogs that were examined after October 16, many young worms were found in the pulmonary arteries and right ventricle of the heart. These findings show that in the Tokyo region the infective larvæ transmitted percutaneously to the dog by the bite of the mosquito grow in unknown sites to a length of 5.0 cm. or more and then migrate to the right ventricle of the heart after mid-October.

Failure to find the intermediate developmental stages of the parasites and their location may be due to the small numbers of worms occurring in the host under natural conditions. The number of worms occurring during one season in different dogs was found to vary between 1 and 21, whilst other dogs were not found to be infected. The average number of worms found in the body of a dog was four, and under these circumstances it would be exceedingly difficult, if not impossible, to find the parasites before they reached the heart. It therefore appeared that artificial inoculation with large numbers of infective larvæ was necessary.

Experiments by Artificial Infection

Nineteen dogs were inoculated artificially with infective larvæ and eight were kept as control animals. The methods of artificial infection were as follows:

Pupæ of *Aedes togoi,* a suitable intermediate host, were gathered and allowed to hatch in the laboratory. The adult mosquitoes were then allowed to suck the blood of dogs carrying microfilariæ. Afterwards the flies were fed until the ingested microfilariæ had grown to the infective stage and were present in the proboscis. The infected flies were then allowed to bite non-infected dogs to produce infection (blood-sucking method), or the infective worm larvæ, which had previously been collected from dissected mosquitoes, were injected subcutaneously or intravenously into non-infected dogs (injection method). The number of infective larvæ inoculated into dogs, which gave positive results, varied between 101 and 2,921. The results of these experiments are shown in Table II.

TABLE II.

Experiment by artificial infection.

No. of experimental dog	Days used for infection	Artificial infection: Number of infective larvæ and method of infection: Blood sucking method	Injections method: Subcutaneous	Injections method: Intravenous	Total of infective larvæ used for infection	Days from artificial infection to autopsy	Number of worms found at autopsy and its location: Intermediate location ♂	Intermediate location ♀	Intermediate location ?	Right ventricle of heart and a. pulmonalis ♂	Right ventricle of heart and a. pulmonalis ♀	Right ventricle of heart and a. pulmonalis ?
1	7		74		74	30 - 23						
Control 1												
Control 2												
2	10	353	212		565	76 - 67	6	3	3	3	5	
3	10	372	234		606	100 - 91	24	21	9	22	21	4
4	17	145	164		309	91 - 75	1	3	3	7	3	1
Control 3										3	1	2
Control 4										1	2	
5	40	1312			1212	279 - 209				33	32	5
6	54		1014		1014	287 - 234				59	76	5
7	5			1171	1171	101 - 97						
8	4		1054		1054	98 - 95	7	11	4	89	48	2
9	27	1131	214		1345	112 - 86	9	14	19	72	56	18
10	8			627	627	116 - 109						
11	7		1164		1164	73 - 67	9	12	6			
12	8	1075			1075	88 - 81	2	6	13	39	19	2
13	23	1900	1021		2921	92 - 70	6	2	3	20	28	
Control 5											5	
Control 6										1	7	
14	1		153		153	80		2	7			
15	1		116		116	85	4	2	5			
16	1		101		101	120				13	16	
17	1		122		122	150				17	37	
18	1		328		328	Not autopsied						
19	1		378		378	Not autopsied						
Control 7												
Control 8												

Intermediate location. Apart from dog No. 1, young worms were found in the submuscular membranes, subcutaneous tissue, fat tissue, subserosæ and muscles of the whole body. The parasites found in these locations, and which had not been seen in naturally infected dogs, were shorter than 5.0 cm., the shortest specimen being 1.1 cm., a size that has not hitherto been recorded in the literature.

Migratory path to the intermediate location. The following three courses are possible: (1) penetration through the tissue, (2) migration through the veins, and (3) migration via the lymphatic system.

(1) Penetration through the tissue. Many young worms were found in intermediate locations of the same side of the body as that artificially infected. As shown in Table III, about 70 per cent of the young worms were found in the

artificially infected left side. Furthermore, with the passage of time the young worms were found in situations more remote from the locus of the infection, as is shown in Table IV. Hence the differences in the distribution of the parasites in the intermediate locations can be interpreted by correlating the number of days after infection occurred with the locus of infection. This shows that the infective larvæ do not migrate through the veins or lymphatic system but by penetration through the tissue.

TABLE III.

Relation between the distribution of young worms in the intermediate locations and infected side.

NO. OF EXPERIMENTAL DOG	ARTIFICIAL INFECTION		LOCUS, NUMBER AND PERCENTAGE OF YOUNG WORMS FOUND AT AUTOPSY					
			LEFT		MEDIAN		RIGHT	
	LOCUS	METHOD	NUMBER	%	NUMBER	%	NUMBER	%
2 3 4	Left cervix and Left abdomen	subcutaneous injection & Blood sucking	90	68.5	2	2.6	21	28.8
8	Left Tarsus	subcutaneous injection	16	72.7	1	4.5	5	22.7
11	Left Tarsus	"	26	70 3			11	29.7
			92	69.7	3	2 3	37	28.0

TABLE IV.

Relation between the distribution of young worms in the intermediate locations and the days from artificial infection by autopsy.

NO. OF EXPERIMENTAL DOG	DAYS FROM ARTIFICIAL INFECTION TO AUTOPSY (AVERAGE)	ARTIFICIAL INFECTION		LOCUS AND NUMBER OF YOUNG WORMS FOUND AT AUTOPSY							
				HIND LIMB		BODY			FORE LIMB		HEAD
		LOCUS	METHOD	LEFT	RIGHT	LEFT	MEDIAN	RIGHT	LEFT	RIGHT	
11	73 - 87(70)	Left Tarsus	subcutaneous injection	14	4	12		7			
8	98 - 95(97)	"	"	2	3	8	10	1	3	8	1

(2) Migration through the veins. As shown in Table II, many young worms were found in dogs injected subcutaneously with infective larvæ, whereas no worms were found in those animals injected intravenously. This clearly demonstrates that infective larvæ do not migrate through the veins.

(3) Migration via the lymphatic system. That this does not occur is based on the following observations. While the infective larvæ of *Metastrongylus elongatus,* a lungworm of the pig, normally migrate through the lymphatic system, causing hæmorrhage in the lymphatic gland, no such pathological change could be detected with our cases of heartworm infections. Furthermore, microscopical examination of that part of the dog's skin bitten by the mosquitoes carrying infective larvæ, showed that the larvæ do not invade the lymphatic system but the corium. The larvæ wander towards various places, some migrating to the corium, others penetrating through the muscular membrane, as shown in Table V.

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TABLE V.

Distribution of the infective larvæ in the tissues of dogs stung by mosquitoes.

NO. OF EXPERIMENT	NUMBER OF MOSQUITOS USED	NUMBER OF MOSQUITOS SUCKED THE BLOOD	HOURS ELAPSED TILL CUTTING OFF THE TISSUE AFTER BLOOD SUCKING.	NUMBERS OF INFECTIVE LARVAE INVADED INTO TISSUE	DISTRIBUTION OF THE INFECTIVE LARVAE IN THE TISSUE: EPITHELION	CORIUM	SUBCUTANEOUS TISSUE	MASCULAR MEMBRANE	MUSCLES
1	2	2	3				)		
2	1	1	2				)		
3	1	1	2				)		
4	2	2	1				)		
5	7	7	48				)		
6	11	11	24	4		——			
7	9	9	4	2		——	)		
8	4	3	6	1		—	)		
9	8	3	19				)		
10	6	2	53				)		
11	7	6	89				)		
12	12	12	5	1		—	)		
13	23	23	5	3		——	)		

—— = Infective larva.
) = Inspected tissue.

TABLE VI.

Distribution and developmental stage of young worms in each intermediate location.

NO. OF DOG	DATE FROM ARTIFICIAL INFECTION TO AUTOPSY (AVERAGE)	LOCATION, NUMBER AND LENGTH OF YOUNG WORMS: SUBMUSCULAR MEMBRANE — NUMB. OF WORMS	SUBMUSCULAR MEMBRANE — LENGTH (cm) (av.)	SUBCUTANEOUS TISSUE — NUMB. OF WORMS	SUBCUTANEOUS TISSUE — LENGTH (cm) (av.)	ADIPOSE TISSUE — NUMB. OF WORMS	ADIPOSE TISSUE — LENGTH (cm) (av.)	SUBSEROSA — NUMB. OF WORMS	SUBSEROSA — LENGTH (cm) (av.)	MUSCLE — NUMBER OF WORMS
11	73 - 67(70)	19	1.1-3.0(2.0)	15	1.2-2.0(1.7)	3	2.0			
2	76- 67(72)	10	1.4-6.0(2.9)	2	2.5					
14	80	7	2.9							
13	92- 70(81)	3	4.0			2	2.5	5	3.5-4.7(3.9)	1
4	91 - 75(83)	4		1	3.5	2	5.0			
15	85	8	3.1-3.9(3.5)	3	2.4-5.0(3.7)					
12	88 - 81(85)	12	3.0-5.2(3.7)					2	4.0-5.0(4.8)	
3	100 - 91(96)	37	2.3-6.3(4.9)	12	3.9-6.5(5.3)	3		2	4.8-5.0(4.9)	
8	98 - 95(97)	13	3.1-5.5(4.4)	5	3.5-6.0(4.5)	2	4.5	2	4.2-5.0(4.6)	
9	112 - 86(99)	22	2.5-9.0(6.2)	6	4.0-11.0(7.0)			4		1
	112 - 67(87)	135	1.1-9.0(4.2)	44	1.2-11.0(4.0)	12	2.0-5.0 (3.5)	15	3.6-5.0(4.3)	2

The correlation between the distribution and the developmental stages of the young worms found in the intermediate locations can be seen in Table VI. In all cases the greatest number of young worms was found in the submuscular membrane, fewer in the subcutaneous tissue, and quite small numbers in the adipose tissue, the subserosa, and the muscle. The growth of the young worms was almost the same in all locations, no difference being observed between males and females.

From the above observations we reached the conclusion that the infective worm larvæ migrate to the intermediate locations by penetration through the tissues.

Migratory Path to the Right Ventricle of the Heart from the Intermediate Location

The following four routes may be considered as a possible migratory path for the young worms in the intermediate location to end their development: (1)

the intermediate location to the heart via the thorax and then via the lung; (2) the intermediate location to the heart via the larynx, the trachea, the bronchi and the pulmonary artery; (3) migration through the lymphatic tissue, and (4) migration through the veins.

Of these four routes (1) and (2) were excluded by the following experiments. It was considered that if the young worms migrate to the heart by routes (1) or (2) they must do so gradually via the thorax or buccal cavity. Our observations had shown that the distribution of the intermediate locations was all over the body and that there was no tendency for the young worms to migrate to the thorax or buccal cavity, as shown in Table VII. Moreover, we had never found young worms in the thorax, pericardium or the bronchi that would indicate the use of route (1) or (2).

TABLE VII.

Distribution of young worms in the intermediate location.

NO. OF TEST DOG	DAYS FROM ARTIFICIAL INFECTION TO AUTOPSY (AVERAGE)	ARTIFICIAL INFECTION			LOCUS AND NUMBER OF YOUNG WORMS FOUND AT AUTOPSY								
		LOCUS	METHOD	NUMBER OF INFECTIVE LARVAE	RIGHT VENTRICLE OF HEART & A. PULMONALIS	INTERMEDIATE LOCATIONS							
						HEAD	NECK	THORAX OUTSIDE	THORAX INSIDE	ABDOMEN OUTSIDE	ABDOMEN INSIDE	FORE LIMB	HIND LIMB
11	73-67(70)	Left hind limb	S.I.	1164				10		9			18
2	76-67(72)	Left abdomen Left hind limb	B.S. S.I.	353 212 565	8	1	1	2		3		2	5
13	92-70(81)	Head Left hind limb	B.S. S.I.	1900 1021 2921	48				1		5	3	2
4	91-75(83)	Left abdomen Left hind limb	B.S. S.I.	145 164 309	11		1	1				1	4
12	88-81(85)	Head	B.S.	1075	60		5		1		1	4	3
3	100-91(95)	Left abdomen Left hind limb	B.S. S.I.	372 274 606	47		2	6		8	2	6	30
8	98-95(97)	Left hind limb	S.I.	1054	139		2	7		4	1	3	5
9	112-86(99)	Head Left hind limb	B.S. S.I.	1131 241 1315	143	1	2	3		4	1	8	12

S.i. = Subcutaneous injection.
B.s. = Blood sucking.

Adult and young worms were collected directly from the heart by dissection and inserted surgically into the subcutaneous tissue of a dog, free from heartworm infection. Young worms were later found in the heart showing that migration to this organ had occurred, as shown in Table VIII. When, however, young worms were inserted into other places, such as the abdominal cavity or the thorax, they did not migrate to the heart but continued their parasitic life in the initial site of implantation. When they were inserted into the trachea they were probably coughed up into the buccal cavity, swallowed and later expelled from the digestive tract since the young worms and worm-like pieces of suture thread, which had been inserted into the trachea, could not later be found anywhere.

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TABLE VIII.

Experiment of insertion of the adult and young worms which were collected from the right ventricle of heart.

EXPERIMENT GROUP	NO. OF DOG	INSERTION: LOCUS AND NUMBER OF WORMS.	AGE (DAYS OLD)	DAYS FROM INSERTION TO AUTOPSY	LOCATION & NUMBER OF WORMS AT AUTOPSY: RIGHT VENTRICLE & A. PULMONALIS ♂	♀	Sex unknown	LOCUS	♂	♀	Sex unknown.	REMARKS
I	1	Subcut- aneous 34 (♂ 15, ♀ 19)	natur. infected Adult worms	7				I.l.	2		1	*
	2	" 5 ♀		10				I.l.		1		—
	3	" 10 (♂ 3, ♀ 7)		12				I.p.	2	3		♂ 2 ♀ 1 alive, 2 dead
	4	" Ca 12		25				I.l.thorax		1	1	*
	5	" 3 (♂ 2, ♀ 1)		30				I.p.	2	1		♀ 1 alive, ♂ 2 dead,
	6	" 14 ♂ 6 ♀ 8	287-234	60				A.c.	1			*
II	1	" 7	108-106	2				I.l.	3	4		—
	2	" 25	120	2				I.l.	11	18		—
	3	" Ca 10	115-114	10				I.l.		3	5	—
	4	" Ca 15	116-115	15	6	1		I.p.		7		* and 7 worms in tha
	5	" Ca 15	115-114	20	5							— place were dead
	6	" Ca 50	112-86	30	20	16	3	I.l.	2			—
III	1	Abdominal cavity 20	100-98	11				A.c.	8			—
	2	" Ca 15	112-86	30				"	10	6		—
	3	" Ca 7	95-85	48				"		1		worms were dead
	4	" Ca 20	111-188	50				"	12	8		—
IV	1	Thorax Ca 7	100-98	8				Thorax	3	4		—
	2	" Ca 20	115-114	20				"	8	9		—
	3	" 5	119-117	41				"	3	2		—
	4	" 5	119-117	48				"	4	2		—
	5	" Ca 7	95-89	50				"	2	4		—
V	1	Trachea 5	115-114	7								—
Control 1		" 5	suture thread	4								—
Control 2		" 10	"	28								—

I.l. = Intermediate location.
I.p. = Inserted place.
A.c. = Abdominal cavity.

* means that the sutured place came to rupture and the inserted worms. wereprobably expelled from the ruptured hole.

These experimental results were sufficient to convince us that the young worms do not migrate to the heart via routes (1) or (2). The fact that the young worms, removed from the final location and transplanted into the intermediate location, can again migrate to the final location is an interesting phenomenon which does not, as yet, seem to have been observed in any other parasitic nematode.

The possible route of migration via the lymphatic system can also be excluded as the normal one for the following reason: In the case of *D. immitis* no pathological changes were found, particularly in the lymph gland, the thoracic canal and cisterna chyli, while in the case of *Metastrongylus elongatus*, the infective larvæ of which migrate through the lymphatic system, remarkable hæmorrhages are always present in the lymphatic system.

The following observations suggest the fourth route, via the veins, to be a possibility: In 6 of 13 dogs artificially infected, 11 young worms were found in the subserosa of the veins and in the adipose tissue surrounding them. No worms were found around the artery. This suggests that the young worms have a taxis to the veins. In another experiment four young worms removed from the intermediate location were inserted into the jugular vein of a dog free from heartworm infection and 17 days later, at autopsy, were found in the right ventricle of the heart. They measured 6.0 (♂), 5.0 (♀), 9.0 (♀) and 11.0 cm. (♀). This demonstrated to us that the young worms migrate from the intermediate location to the right ventricle of the heart when they penetrate the veins. The migration from the intermediate locations to the heart is made between the 85th and the 120th day after infection since young worms were never found in the heart or pulmonary artery of dogs dissected within 85 days from infection; nor were they found in the intermediate locations of dogs dissected later than 120 days after infection (see Table IX).

TABLE IX.

Localisation of young worms in the intermediate location and right ventricle of heart (arranged after the number of days from artificial infection to autopsy).

NO. OF EXPERIMENTAL DOG	NUMBER OF INFECTIVE LARVAE ARTIFICIALLY INJECTED.	DAYS ELAPSED TO AUTOPSY AFTER INFECTION	NUMBER OF YOUNG WORMS FOUND AT AUTOPSY								REMARKS
			TOTAL	INTERMEDIATE LOCATIONS				RIGHT VENTRICLE OF HEART & A. PULMO.			
				TOTAL	♂	♀	SEX UNKNOWN	TOTAL	♂	♀	SEX UNKNOWN
11	1164	73 - 67	37	37	9	12	6				Prevented natural infection
14	153	80	7	7		2	5				—•—
15	116	85	11	11	4	2	5				—•—
12	1075	88 - 81	81	21	2	6	13	60	39	19	2
4	309	91 - 75	18	7	1	3	3	11	7	3	1
13	2921	92 - 70	59	11	6	2	3	48	20	28	
8	1054	98 - 95	161	22	7	11	4	139	89	48	2
3	606	100 - 91	101	54	24	21	9	47	22	21	4
9	1345	112 - 86	176	33	9	14	10	143	72	53	18
16	101	120	29					29	13	16	—•—
17	122	160	54					54	17	37	—•—
5	1312	279 - 209	73					73	33	35	5
6	1014	267 - 234	140					140	59	76	5

The length of migrating worms seen in these experiments varied between 3.2 cm. (the shortest specimen found in the heart) and 11.0 cm. (the longest specimen found in the intermediate location).

Discussion and Conclusions

In the final host the life-cycle of all species of the Filariidæ has hitherto remained unknown; even in the case of *Dirofilaria immitis* there has been no knowledge of the development of the parasite from the time it is transmitted percutaneously by the bite of the mosquito, and when it measures only about 1.0 mm. long, until it is found in the heart and pulmonary artery of the dog where, in its normal location, it measures more than 5.0 cm. long.

Attempts to solve the problem by examining naturally infected dogs were unsuccessful and observations on dogs artificially infected with hundreds of infective larvæ were then made. In the experimental dogs it was possible to find a large number of young worms in the submuscular membrane, subcutaneous tissue, adipose tissue, subserosa and muscles. These tissues served as intermediate locations before migration to the right ventricle of the heart occurred. The parasites in these locations were generally so small that they had escaped detection in the right ventricle and pulmonary artery. Having determined the intermediate locations, three possible routes of migration from the point of entry into the host to these locations were investigated. It was then found that the infective larvæ do not migrate through the veins or through the lymphatic system but penetrate through the tissue, to reach the intermediate locations where they develop normally.

Four possible routes of migration from the intermediate locations to the right ventricle of the heart were then investigated. No migration appeared to occur via the thorax and lungs, or via the buccal cavity, larynx, trachea, bronchi and pulmonary artery or via the lymphatic system but it did occur via the veins between 85 and 120 days after infection, the parasites measuring between 3.2 and 11.0 cm.

REFERENCES

Bancroft, T. L. (1903): *J. roy. Soc., N.S.W., 37,* 254.
Fülleborn, F. (1907): *Münch. med. Wschr., 54* (10), 497.
Fülleborn, F. (1908): *Arch. Schiffs—u. Tropenhyg., 12,* 313.
Grassi, G. B., and Noé, G. (1900): *Zbl. Bakt., 1 Abt., 28* (19), 652.
Inoue, T. (1936): *Keio Igaku, Tokyo, 16,* 1737.
Inoue, T. (1938): *Idem, 18,* 71.
Manson, P. (1877): *Med. Rep., Shanghai, 13.*
Manson, P. (1878): *Idem, 14,* 1.
Manson, P. (1884): *Trans. Linn, Soc. Lond. (Zool.),* 2s., *2* (10), 367.
del Rosario, F. (1936): *Philipp. J. Sci., 60,* (1), 45.

EXPERIMENTS WITH MYXOMATOSIS IN THE HEBRIDES

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FOLLOWING the reports on the successful use of myxomatosis virus in Australia several requests for a supply of virus were received from sheep farmers whose grazings were being destroyed by rabbits. In view of the widespread damage being done it was felt that an investigation into the use of myxomatosis would be of some value. Inquiries were made to find a suitable isolated island for the purpose and eventually the Heisker or Monach Islands were selected. These islands are situated in the Atlantic ten miles west of Benbecula and seven miles south-west of the nearest point on North Uist. Of the five islands only three have reasonable grazing. Altogether they cover about 1,000 acres, 600 being grass and the remainder sand dunes with grazing in the hollows.

Ceann Ear or East End is the largest island, Shivinish, the middle island, is the smallest and Ceann Iar or West End is second in size. They are joined to each other by sand bars which are uncovered at low tide, and at low tide the stock can and do go from one island to the other. Until the 1930's the islands were inhabited by a colony of about 100 people who lived by fishing and agriculture. There were about 1,000 head of stock on the islands then and rabbits, which had been introduced early in the century, were kept under control by the inhabitants. Depopulation was almost complete in 1939, one family remaining until about 1945. By 1951 rabbits had overrun the islands and the tenant could only graze 25 young cattle and 50 sheep. There were no predatory animals on the islands apart from four cats although no doubt black-backed gulls and ravens did prey on the young rabbits. Lobster fishermen from the North Uist spend the summer months based on the Heiskers but the number of rabbits they shoot is insignificant. Rabbits have, therefore, a more or less free run of the islands and at the time of our first visit in early July, 1952, they literally swarmed over them. The best grazing is on the East End and there were noticeably more rabbits on it than on the other two.

Published information indicated that the disease was transmitted by biting insects and it was assumed that because biting insects can be a plague in the Hebrides, the Heiskers would be no exception. It was assumed, too, that biting insects would be absent during the winter and that the disease would, therefore, probably die out then. We aimed to introduce as much infection as possible into the rabbits on the islands in midsummer in the hope that it would be widely

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spread by the autumn. The idea of ferreting and netting wild rabbits and infecting them by injection was discarded for two reasons, the first because in the rabbits' breeding season ferrets do not work very well; the second because it would have been necessary for us to spend several days at a time on the islands in order to trap sufficient rabbits. It was decided to take batches of laboratory rabbits to the islands and infect them by the injection of virus prior to their release at selected heavily populated warrens. That could be done during a visit to the islands of only a few hours.

The first visit was made on July 8, 1952, when 12 rabbits were released immediately after being infected. Further visits with 12 rabbits were made on July 15, 22 and 29. During these later visits, which lasted only a few hours, no rabbits were seen affected with myxomatosis, not even our own laboratory rabbits. For controls we injected rabbits in the laboratory with each batch of injection material and were able to reproduce the disease satisfactorily. A final visit for the year was made on September 11, 1952, when again 12 laboratory rabbits were injected and released. At that visit none of our previously injected laboratory rabbits, recognisable because of their colour, were found. It was considered that there were fewer rabbits at one end of the East End but that the rabbit population in general had shown little if any reduction. No rabbits were seen showing symptoms of myxomatosis. Obviously our attempt to introduce a heavy infection had failed. In all, 48 infected rabbits had been released in July and it was fully anticipated that that number on selected sites should have been sufficient to set up an infection. No satisfactory explanation could be given apart from the possibility of lack of carriers and it had been noticed that none of us had been bitten by insects during our short periods on the islands. It was decided to repeat the investigation in the spring of the following year before abandoning it completely.

During the winter months the tenant was able to visit the islands occasionally and towards the end of March, 1953, he reported no change in the rabbit population. But during the period of a month from the time of his last visit until our first for the year on April 29, 1953, a very considerable reduction, estimated to be about 60 per cent, was noticed particularly in the East End. At that visit ten infected rabbits were introduced. Although again none of the wild rabbits were seen affected, it seemed probable that myxomatosis was responsible for the reduction in numbers. Assuming that myxomatosis was the cause, the virus then had survived the winter probably in some biting insect or other carrier since direct contact transmission from rabbit to rabbit would have caused a gradual reduction in numbers over the winter months. The sudden reduction in the rabbit population could be accounted for if the rabbit flea carried over the infection during the winter and reproduced in large numbers in the early spring. It is well known that the rabbit flea exists wherever there are wild rabbits but its life cycle is not known nor was it known that it could transmit myxomatosis. Investigations in the laboratory confirmed that the rabbit flea could transmit the disease—confirmed later by Lockley (1954). On several occasions a healthy

rabbit was infected by placing on it fleas taken from a rabbit which had died from the disease. Also a suspension of ground-up fleas from an infected rabbit produced the disease when injected into a healthy rabbit. In addition, in a series of observations lasting over six months two dustbins were used. One bin was infested with fleas, the other not. Initially an infected rabbit was placed in each bin and thereafter within 24 hours of each rabbit dying it was replaced by a healthy rabbit. The infection was kept active by this means for over six months before it was discontinued and it was noticed that the rabbits in the flea-infested bin developed the disease fairly consistently 48 hours earlier than in the non-flea bin.

On May 20, 1953, another visit to the Heiskers was made with ten laboratory rabbits. On this occasion R. Allan accompanied us and remained alone on the islands for six days carrying out a survey of the insect population. During his stay he saw our laboratory rabbits develop myxomatosis and was able to observe that they fraternised with the wild rabbits and used their burrows. There was, therefore, ample evidence that our laboratory rabbits could be a source of contact infection for the wild rabbits and that by using the burrows the infection could be spread by fleas. His careful survey failed to show any evidence of biting insects other than fleas, not even in the proximity of the two small lochs on the islands.

No further visits were made during the year until December 5 when one of us paid a short visit when it was found that there was no obvious change since the spring visits.

A visit in early April, 1954, had to be abandoned because of gales and high seas, and postponed until May 13 when an opportunity was taken to spend nine days on the islands. We were accompanied on this occasion by H. V. Thompson of the Infestation Control Research Division of the Ministry of Agriculture. No rabbits were infected. Six sites were selected, four on the East End and two on Shivinish and rabbits in defined areas from these points were counted at sunrise and sunset on three successive days. The weather was perfect for the purpose and the counts of rabbit numbers can be accepted as fairly accurate. A number of rabbits were trapped and shot and blood samples from them sent to Hudson at Weybridge for examination for myxomatosis antibodies. All proved negative. No rabbits affected with myxomatosis were seen nor were any seen which showed evidence of having recovered.

A second visit lasting nine days was made towards the end of September, 1954. Rabbit counting from the same sites was carried out. This time the weather was unfavourable with gales and storms. On several occasions the counting had to be abandoned because of the small number of rabbits out grazing. The counts obtained were therefore probably less than the actual number of rabbits in the area. In spite of that, as will be seen in the table, the rabbit population had increased on all but one of the six sites. It is of interest to note that the site showing the decrease is the one where most infected rabbits were released.

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TABLE

Site	May Maximum	September Maximum
1	18	38
2	95	72
3	40	63
4	30	38
5	35	57
6	28	57
Total average	41	54

It was interesting to observe, too, that the rabbit population on the West End had noticeably increased and a count from a site there at midday on three days (not consecutive) gave a maximum count of 150. Owing to the tides it was impossible to make a count there in the morning or evening. It was also of interest to note that there was no evidence that the cattle and sheep on the islands had been in the West End for some time probably due to the fact that the rabbits had eaten down the grass and fouled it. Rabbits were again shot and blood samples collected and sent to Weybridge. Hudson again reported the absence of myxomatosis antibodies. No rabbits affected with the disease were seen and with the rabbit population increasing it was considered certain that the disease had died out.

Discussion

Laboratory rabbits were taken to the Heiskers on eight occasions and in all 80 rabbits were released, the majority on the East End but some on Shivinish and the West End. Before release the rabbits were injected with known infective material. During the course of the investigation the laboratory rabbits were seen showing symptoms of myxomatosis and were also seen mixing with the wild rabbits and using their burrows. At no time, however, were the wild rabbits on the Heiskers seen showing symptoms of the disease. In the autumn following the first introduction it was thought that the number of rabbits had decreased at the east end of the East End, but it was not until the following spring that a very marked reduction in the rabbit population took place all over the East End. No noticeable decrease in the rabbit numbers occurred on Shivinish or the West End nor was any further decrease in the number of rabbits observed on any of the three islands thereafter. Indeed, at the final visit rabbit counting indicated a definite increase in the population during 1954.

Although no wild rabbits were seen affected with myxomatosis it is confidently accepted for the following reasons that myxomatosis did break out and cause the deaths of large numbers of rabbits. (1) The experimental rabbits did develop the disease and they were seen using the burrows and mixing with the wild rabbits, thus providing ample opportunity for direct contact infection.

(2) More experimental rabbits were released on the East End than on the other two islands and it was on the East End that the spectacular decrease in the rabbit population occurred. (3) The decrease in rabbits on the East End may have been due by coincidence to some other disease but since no dead or dying rabbits were seen we have no proof of the existence of an outbreak of an intercurrent disease and the most likely cause of death can be assumed to have been myxomatosis. A post-mortem examination was carried out on upwards of 100 rabbits shot in 1954 and about 40 per cent showed lesions of Coccidiosis. Affected rabbits were equally distributed over the three islands, thus lending support to the inference that myxomatosis must have been the cause of the reduction in the rabbit population on the East End. (4) Dying animals in the Heiskers are attacked by gulls and ravens. During the spring visit in 1954 a ewe was noticed one night to be seriously ill. Next morning she was found dead with most of the soft tissues of the head pecked away. A post-mortem examination was carried out and within 48 hours very little remained of the carcase but wool and bones. It can be safely assumed that sick rabbits are quickly spotted and attacked and that could account for our inability to observe any wild rabbits showing symptoms. It is quite probable that some if not all our experimental rabbits died in that way. Many rabbit carcases were seen.

A survey by an entomologist failed to reveal any biting insects on the Heiskers, apart from the rabbit flea, which could assist in the spread of the disease, and in the laboratory it was confirmed that the rabbit flea could transmit the disease.

The grazing on the Heiskers, which could originally carry up to 1,000 head of cattle and sheep, was reduced in 1952 to 25 cattle and 50 sheep. Resulting from the decrease in rabbits on the East End in 1953 the stock was increased to over 50 cattle and over 80 sheep in 1954. Many of the burrows in 1953 were obviously not being used and grass was growing through the sand scraped out by the rabbits. By 1954 these burrows were again active and the grass had disappeared around them.

Further observations have shown in the laboratory that the sheep tick, *Ixodes ricinus,* can also be a transmitter of myxomatosis. Whether the virus will be able to survive longer in the sheep tick than in the flea is not yet known; certainly the tick will survive off its host for much longer than the flea.

Attempts to transmit the disease by the harvest mite have failed .

Conclusions and Summary

Myxomatosis introduced to the Heisker islands which were overrun with rabbits failed to cause a widespread outbreak of the disease due, probably, to the lack of insect vectors. For reasons given, however, the disease was assumed to have survived the winter and caused heavy mortality on one island and it was considered likely that rabbit fleas were responsible for the survival of the disease over the winter. Following a drastic reduction in the rabbit population the disease died out. It can be surmised, therefore, that in the absence of biting insects which fly there has to be a certain minimum rabbit population before

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fleas can keep the infection alive. Consequently it can be deduced that during the winter when there are no biting insects apart from fleas it is fairly certain that the disease will die out if an area has been severely depleted of wild rabbits. Rabbits surviving will breed during the ensuing year and unless fresh infection is introduced the rabbit population will quickly increase. Applying that reasoning to the outbreaks in Britain it is suggested that the disease, after causing a very considerable reduction in the rabbit population, will eventually disappear unless fresh infection is introduced from time to time.

Acknowledgments

Our thanks are due to many people who assisted in the investigations but particularly to R. McAuley, the tenant of the Heiskers; to D. S. Shaw, factor for the North Uist Estate; to D. Kerr, the local agricultural advisory officer of the North of Scotland College; and to Dr. Weston Hurst, of I.C.I., who supplied the strain of myxomatosis virus.

REFERENCE

Lockley, R. M. (1954): *Vet. Rec., 66,* 434.

STUDIES ON HÆMORRHAGIC SEPTICÆMIA OF CATTLE
III. Production of Adjuvant Vaccine*

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Introduction

WATER-IN-OIL emulsion adjuvant vaccines are well known and the vaccine described in this paper was designed as an improvement on the existing prophylactics against hæmorrhagic septicæmia.

Strain of Pasteurella Used

It is apparent that the only type of pasteurella of importance in hæmorrhagic septicæmia vaccines is *Pasteurella multocida* type I (see Roberts 1947, Bain 1954, Hudson 1954). The "Insein" strain of *P. multocida* which was isolated in Burma in 1952 is highly virulent for cattle by injection and remains in the fluorescent phase for several subcultivations after recovery from an experimental animal. It has no other special features and any strain of *P. multocida* type I with similar gross characteristics would probably serve as well. The "Insein" strain which has been used mainly in this work survives in infected blood for six months or more when stored at —20° C. Lyophilized infected blood is also a good source of virulent capsulated pasteurellas. The type I strains dissociate readily on artificial media and in 3 to 12 transfers, depending on the medium and the strain, the original fluorescent phase is replaced by the blue phase. The

* Assisted by a grant from the University of Sydney Research Fund.

shift in phase is not always obvious as on some media it is impossible to distinguish between the fluorescent and blue phases. The most satisfactory medium for distinguishing them easily by eye is tryptose agar. A subsequent paper describes how the yield of antigens differs in the two phases.

Growth of Bacteria

The pasteurellas can be grown on agar or in broth although for large-scale production broth has many advantages.

(*a*) *Agar Method.* Several kinds of media containing meat extract or infusion, yeast extract, peptone, sodium chloride and a little glucose (from 0.1 per cent to 0.5 per cent) have been used satisfactorily; the peptones included Merck's, Difco Tryptose and Neo-peptone. A good cheap medium for large-scale production is a modification of the Ward and Rudd medium solidified with agar. The selected medium is distributed in 1,000 ml. Roux flasks. The strength of the agar gel should be sufficiently high to allow expeditious handling of the flasks and about 2 per cent is adequate for most brands. When the medium has set properly each flask is seeded with 1.5 ml. of a six hour broth culture, prepared from selected colonies grown on an agar plate which has been sown with infected blood. A semi-automatic syringe is useful for seeding.

After 20 hours' incubation at 37° C. the flasks are inspected for purity and satisfactory growths are washed off with glass beads and a small volume of 0.25 per cent formol saline. The dense suspension is filtered through gauze, checked microscopically and standardised to the opacity of Brown's tube No. 7 by diluting with formol saline. The yield is usually 40 to 50 bovine-doses per 1,000 c.c. flask, each flask containing 180 to 200 ml. medium; in other words, 200 to 250 doses per litre of medium used. The standardised suspension is used for blending when it has passed sterility tests.

(*b*) *Broth Method.* The density required in the bacterial suspension to keep the dose to a reasonable level cannot be attained in ordinary flasks of broth as this rarely exceeds the density of Brown's tube No. 2 after 24 hours' incubation. Concentration by centrifugation, even in a continuous-flow centrifuge, is not practicable for large-scale production. Fortunately, by aerating broth cultures it is possible to achieve densities up to Brown's tube No. 7 with only 20 hours' incubation and cells so produced are capable of giving rise to protective antibodies in cattle.

The medium is a slight modification of one described by Ward and Rudd (1938). The ingredients are few and simple and the yield of bacteria equal to or better than that from any other medium tried. The modified formula is as follows:—

Beef muscle	400 gm.
Disodium phosphate ...	0.5 gm.
Water	1,000 ml.

Adjust *pH* to 9.0 with N/1 NaOH. Heat to 79-80° C. for five minutes. Cool to 45° C.. add 2.0 gm. trypsin suspended in 10 ml. saline, incubate 45 minutes in a 45° C. waterbath. Adjust to *pH* 6.0 with N/1 HCl and boil for 20

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fleas can keep the infection alive. Consequently it can be deduced that during the winter when there are no biting insects apart from fleas it is fairly certain that the disease will die out if an area has been severely depleted of wild rabbits. Rabbits surviving will breed during the ensuing year and unless fresh infection is introduced the rabbit population will quickly increase. Applying that reasoning to the outbreaks in Britain it is suggested that the disease, after causing a very considerable reduction in the rabbit population, will eventually disappear unless fresh infection is introduced from time to time.

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Introduction

WATER-IN-OIL emulsion adjuvant vaccines are well known and the vaccine described in this paper was designed as an improvement on the existing prophylactics against hæmorrhagic septicæmia.

Strain of Pasteurella Used

It is apparent that the only type of pasteurella of importance in hæmorrhagic septicæmia vaccines is *Pasteurella multocida* type I (see Roberts 1947, Bain 1954, Hudson 1954). The " Insein " strain of *P. multocida* which was isolated in Burma in 1952 is highly virulent for cattle by injection and remains in the fluorescent phase for several subcultivations after recovery from an experimental animal. It has no other special features and any strain of *P. multocida* type I with similar gross characteristics would probably serve as well. The " Insein " strain which has been used mainly in this work survives in infected blood for six months or more when stored at —20° C. Lyophilized infected blood is also a good source of virulent capsulated pasteurellas. The type I strains dissociate readily on artificial media and in 3 to 12 transfers, depending on the medium and the strain, the original fluorescent phase is replaced by the blue phase. The

* Assisted by a grant from the University of Sydney Research Fund.

shift in phase is not always obvious as on some media it is impossible to distinguish between the fluorescent and blue phases. The most satisfactory medium for distinguishing them easily by eye is tryptose agar. A subsequent paper describes how the yield of antigens differs in the two phases.

Growth of Bacteria

The pasteurellas can be grown on agar or in broth although for large-scale production broth has many advantages.

(*a*) *Agar Method.* Several kinds of media containing meat extract or infusion, yeast extract, peptone, sodium chloride and a little glucose (from 0.1 per cent to 0.5 per cent) have been used satisfactorily; the peptones included Merck's, Difco Tryptose and Neo-peptone. A good cheap medium for large-scale production is a modification of the Ward and Rudd medium solidified with agar. The selected medium is distributed in 1,000 ml. Roux flasks. The strength of the agar gel should be sufficiently high to allow expeditious handling of the flasks and about 2 per cent is adequate for most brands. When the medium has set properly each flask is seeded with 1.5 ml. of a six hour broth culture, prepared from selected colonies grown on an agar plate which has been sown with infected blood. A semi-automatic syringe is useful for seeding.

After 20 hours' incubation at 37° C. the flasks are inspected for purity and satisfactory growths are washed off with glass beads and a small volume of 0.25 per cent formol saline. The dense suspension is filtered through gauze, checked microscopically and standardised to the opacity of Brown's tube No. 7 by diluting with formol saline. The yield is usually 40 to 50 bovine-doses per 1,000 c.c. flask, each flask containing 180 to 200 ml. medium; in other words, 200 to 250 doses per litre of medium used. The standardised suspension is used for blending when it has passed sterility tests.

(*b*) *Broth Method.* The density required in the bacterial suspension to keep the dose to a reasonable level cannot be attained in ordinary flasks of broth as this rarely exceeds the density of Brown's tube No. 2 after 24 hours' incubation. Concentration by centrifugation, even in a continuous-flow centrifuge, is not practicable for large-scale production. Fortunately, by aerating broth cultures it is possible to achieve densities up to Brown's tube No. 7 with only 20 hours' incubation and cells so produced are capable of giving rise to protective antibodies in cattle.

The medium is a slight modification of one described by Ward and Rudd (1938). The ingredients are few and simple and the yield of bacteria equal to or better than that from any other medium tried. The modified formula is as follows:—

Beef muscle	400 gm.
Disodium phosphate ...	0.5 gm.
Water	1,000 ml.

Adjust *pH* to 9.0 with N/1 NaOH. Heat to 79-80° C. for five minutes. Cool to 45° C., add 2.0 gm. trypsin suspended in 10 ml. saline, incubate 45 minutes in a 45° C. waterbath. Adjust to *pH* 6.0 with N/1 HCl and boil for 20

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minutes. Filter, cool and adjust to *pH* 7.8. Add $CaCl_2$ to 0.13 per cent and NaCl to 0.25 per cent. Boil for five minutes and filter. Add 0.1 yeast extract. Sterilise.

Aeration can be applied through a suction pump or by positive pressure from an air compressor. The latter is preferred as it reduces the risks of contamination of the culture. The air bubbles must be distributed finely through sintered glass gas distributors or through earthenware filter candles. Air pressures required vary with the equipment; for a Berkefeld "N" filter candle, 18 lb. per square inch was needed. A thick stream of fine bubbles is the aim, producing a fine dense froth.

Control of frothing is a problem in home-made aeration jars. Antifrothing agents such as alcohols are toxic to the bacteria and inhibit growth. A small quantity of linseed oil (1 ml. per 10 litres) limits frothing but also causes decapsulation of the bacteria. Silicones have not been tried adequately. Mechanical froth breakers are impractical in small jars but effective in large tanks. For small jars containing 10 litres of broth, a second jar acting as a trap in the outlet is the best solution. The total loss of culture is slight, about half a litre in 20 hours.

For production of vaccine on a moderate scale, home-made aeration jars are adequate. Twenty-litre pyrex bottles are most suitable with rubber bungs bored for air inlet, outlet and seeding tube. A smaller bottle of about 5 litres capacity is placed in the air outlet as a froth trap. About ten litres of broth are sterilised in the bottle which has been fitted with gas distributors or filter candles. The bottle is seeded through a suitable intake with 200 ml. of a six hour broth culture prepared as described above for the agar method. It is incubated at 37° C. and two hours after seeding aeration is started. Twenty hours later the culture is checked for density and purity and formalin is added to a concentration of 0.25 per cent. The culture may be fed at about the twelfth hour of incubation with peptone solution enough to give a concentration of 1 per cent. As there is very little fermentable carbohydrate in the medium the *pH* remains almost unchanged.

The density attained in crude apparatus such as is described above, varies from that of Brown's tube 5 to tube 7. In our hands, the density of pooled batches is usually a little below the tube 7 standard required for direct emulsifying. The deficiency can be made good by centrifuging a small quantity of culture and recovering the bacteria. When the culture is formalinised, standardised and passed for sterility it can be blended with oil.

Emulsification

The oil used is a light mineral oil and in all experiments conducted so far the liquid paraffin "Ondina 17" supplied by the Shell company has been found most suitable. Lanoline (Adeps lanæ anhydros B.P.) has been adopted as the stabiliser, not because it is necessarily the best but because it is readily available in all countries. The method of mixing for small-scale laboratory experimental work has already been described (Bain, 1954). The aim is to produce a stable

water-in-oil type of emulsion with the bacterial suspension as the disperse phase. For the peculiarities of emulsions those interested should consult a textbook such as " Clayton's Theory of Emulsions " (Ed. Sumner 4th Edn. Churchill London).

For the suspension prepared from agar, a formula of 15 parts bacterial suspension, 10 parts oil and 1½ parts lanoline may be adequate to produce a good emulsion. It may be found necessary to raise, by a small amount, the proportion of oil and lanoline. For the broth-grown suspension a " fatter " formula is quite definitely needed, i.e., 15 parts suspension, 12 parts oil, 2 parts lanoline. The lanoline is calculated as parts by volume of melted lanoline.

The amount of oil and lanoline needed is computed from the quantity of bacterial suspension according to the formula. After sterilisation all the oil and lanoline is placed in a vessel such as a 10-gallon milk can, the emulsifier is started and the broth added steadily over a period of 10 minutes. Mixing is continued for another five minutes. Next morning each batch is blended again for 5 to 10 minutes. An emulsifier found to be highly satisfactory is the Australian " Earnshaw" turbo-emulsifier consisting of a stator and rotor head with vanes which produce a shearing action. The rotor is driven by a shaft at 1,200 r.p.m. The apparatus is portable and can be clamped above any suitable container. Emulsions produced as described above have remained stable for the past year. The emulsion should be fine and smooth without any globules of disperse phase visible to the naked eye. A little creaming down on standing is normal with separation of a little clear oily continuous phase on the top. Creaming in the opposite direction indicates an incomplete or an inverted emulsion which is quite useless as an adjuvant.

The minimum recommended dose for the above mixture is 3 ml. given intramuscularly. The cost of materials is trifling; in Australia 10 ml. costs approximately one penny.

Addition of Mycobacterium

It is well known from the work of Freund, Kabat and others that the presence of killed mycobacteria in the oily phase enhances the antigenicity of certain substances, particularly proteins. Some experiments with *Mycobacterium butyricum* added to *P. multocida* adjuvant vaccine are in progress now using 0.1 per cent dried autoclaved *M. butyricum* in the oil. No particular benefit from this has been noted in mice and although the experiment in cattle is not finished, there is not in the early stages any evidence for a higher level of circulating antibody in the groups receiving mycobacteria.

Keeping Properties

This has not been fully tested. A small sample of agar-grown adjuvant vaccine plus mycobacteria brought from Thailand to Australia produced an excellent antibody response in five cattle ten weeks later.

Discussion

From conventional broth-grown suspensions the yield is 100 to 200 doses per litre. From aerated broth, 500 doses per litre of adjuvant vaccine can be

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obtained provided the density is adequate. Even if the aerated broth is not made into adjuvant vaccine it is a more economical vaccine than one produced by ordinary still-culture methods. If the cultures are grown as described, no detectable dissociation occurs and the aerated cells are capable of stimulating formation of circulating antibodies in cattle.

The Ward and Rudd medium is cheap and effective. For large-scale production of vaccine a digester tank and centrifugal filter would be needed to cope with the large volumes.

A production tank for vaccines of this aerated type has been described by Heden, *et al.* (1952). It is a stainless steel tank with a range of fitments for sterilising, incubating, seeding, feeding and recording results on the culture *in situ*. Frothing is controlled mechanically. Although such a tank has not been tried here it appears to be an ideal arrangement for production of 100 to 200 litres at a time.

Summary

Methods are described for the production of hæmorrhagic septicæmia oil adjuvant vaccine. By these methods a modestly-equipped laboratory could produce enough vaccine for extensive field trials.

REFERENCES

Bain, R. V. S. (1954) : *Bull. Off. int. Epiz., 42,* 256.
Freund, J. (1947) : *Ann. Rev. Microbiol., 1,* 291.
Heden, C. G., Malmgren, B., Sundstrom, K. E., and Tornquist, B. (1952) : *Acta Path. Microbiol. Scand., 30,* 284.
Hudson, J. R. (1954) : *Bull. Off. int. Epiz., 42.*
Roberts, R. S. (1947) : *J. comp. Path., 57,* 261.
Ward, H. K., and Rudd, G. V. (1938) : *Aust. J. exp. Biol. Med. Sc., 16,* 181.

SOME OBSERVATIONS ON THE EFFECTS OF ENVIRONMENT ON MICE DOSED WITH DINITRO-ORTHO-CRESOL

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Dinitro-ortho-cresol had been used as a selective weed-killer in cereal crops, insecticide, ovicide and fungicide. Dodds and Robertson (1933) claimed that it gave favourable results in cases of obesity. Murray and Mark (1935) reported a case of poisoning in a girl during her treatment with DNOC tablets for slimming. Bidstrup and Payne (1951) reviewed the literature on DNOC and believed that DNOC might cause death to the operator on using it as a crop spray. They recorded that fatal cases had been reported in various countries when DNOC was used when the weather was unusually hot. Bidstrup (1953) referred to the occurrence of fatal poisoning from DNOC amongst animals in contact with the crop spray operation.

The object of the present work is to estimate the minimum lethal dose of the DNOC in mice, and to investigate the effect of the temperature and humidity on mice given a minimum lethal dose.

Material and Technique

Albino mice of both sexes, six weeks old and of average weight of 25 gm. each, were used.

DNOC was used in a 0.1 per cent solution which was made by dissolving its crystalline form in physiological saline. Mice were injected intraperitoneally with doses varying from 20-30 mg. per kilo body weight, according to the experiment.

Experimental

A—Determination of the M.L.D. of DNOC in Mice

Experiment I: Fifty albino mice divided into five equal groups were used. Mice in each group were injected intraperitoneally with doses of 20, 22.5, 25, 27.5 and 30 mg. per kilo body weight respectively. Groups of animals after being injected, were kept for 48 hours under normal conditions of 18° C. temp. and 28 per cent relative humidity. Animals which died during the experiment were recorded. The results are summarised in the following table:—

TABLE I

The percentage mortality in each group of animal injected with DNOC. with DNOC

Group No.	Dose of DNOC per kilo body wt.	No. of mice died out of 10 Time in hours after dosing ½	1	1.5	2	3	4	5	6	No. of total losses	% of total mortality
I	20 mg.	—	—	—	—	—	—	—	—	—	0%
II	22.5 mg.	1	—	—	—	—	—	—	—	1	10%
III	25 mg.	2	1	2	—	—	—	—	—	5	50%
IV	27.5 mg.	4	2	1	2	—	—	—	—	9	90%
V	30 mg.	6	2	2	—	—	—	—	—	10	100%

From the above results shown in Table I, a graph was made by plotting the PROBIT against the LOG doses. From this graph the M.L.D. was calculated and found to be 25 mg. per kg. of body weight.

Experiment II. For further confirmation, another fifty albino mice were injected intraperitoneally with 25 mg./kg. (M.L.D.) and were kept under the same conditions as Experiment I. Twenty-five mice died within three hours after injection, while the other 25 mice survived.

B—Effect of temperature and humidity on mice dosed with the M.L.D. of DNOC.

Eighty albino mice divided into eight equal groups were used in the following experiments in which the procedure and technique were the same.

Experiment I—Short period of acclimatisation.

(A) The first group, which served as control, was injected intraperitoneally with the M.L.D. of DNOC which had been determined previously. The animals were then kept in the cabinet in a separate cage for six hours.

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The second group was preconditioned to a new environment (i.e., temperature 35° to 37° C. and relative humidity 28 per cent to 30 per cent) for 18 hours. They were then injected intraperitoneally with the M.L.D. of DNOC and kept in a separate cage together with the first group in the cabinet at the same temperature and relative humidity for six hours. During the time of exposure, the results were recorded. It was found that the total mortality was 80 per cent in the first and 65 per cent in the second groups.

(B) In this experiment the temperature of the cabinet and the preconditioning time remained unchanged, i.e., 35° to 37° C., while the relative humidity was raised to 58°—60°.

Two groups of mice were used as in experiment (A). The result recorded at the end of the experiment showed a 95 per cent final mortality in the control group and 70 per cent in the preconditioned group.

Experiment II—Prolonged period of acclimatisation

(A) The temperature and relative humidity of the cabinet in this experiment was 35° to 37° C. and 58 per cent to 60 per cent respectively.

The first group of mice was considered as a control. The second group after being preconditioned to the artificial environment of the cabinet for seven days, was then injected with the M.L.D. of DNOC.

The mortality in the preconditioned group was found to be 65 per cent at the end of the experiment, while in the non-preconditioned one it was 80 per cent.

(B) In this experiment, both the temperature and preconditioned exposure time were the same as in Experiment II (A), while the relative humidity dropped to 28°—30°.

The mortality of the preconditioned group was 50 per cent, while in the control it was 80 per cent.

Conclusion

From the present work the following conclusions could be drawn :—

1. The temperature and relative humidity had a great influence on the toxicity of DNOC. This supports the views pointed out by some other workers who gave evidence to the losses in animals and men during spraying crops, specially in hot weather.

2. It was also noted that a short period of exposure to hot weather lowers body resistance and consequently the mortality rate. On the other hand prolonged exposure acclimatises animals to hot weather and therefore lowers the percentage of deaths.

3. The characteristic symptoms of DNOC poisoning were excessive sweating, thirst, rapid respiration, anxiety and restlessness.

4. Post-mortem lividity at the neck region of the animal was noticed immediately after death; rigor mortis was, moreover, well marked in the back. At necropsy, the chief findings were the unclotted blood and the deep yellow coloration of urine retained in the urinary bladder.

Acknowledgment

I am indebted to the staff of the Department for Research in Industrial Medicine, Medical Research Council Laboratories, London, for their assistance and useful suggestions.

REFERENCES

Bidstrup, P. L. (1953): Personal communication, London.
Bidstrup, P. L., and Payne, D. J. H. (1951): *Brit. med. J.*, *2*, 16.
Dodds, E. C., and Robertson, J. D. (1933): *Lancet*, *2*, 1137.
Murray, B. G., and Mark, J. W. (1935): *Ann. intern. Med.*, *9*, 198.

ABSTRACT

FASCIOLA GIGANTICA IN TURKEY. "Hatay'in Kirikhan Ilçesinde Görülen Fasciola Gigantica'dan Mütevellit Distomatoz Salgini Hakkinda." *Enver Can and Dr. Yahya Tamer, "Türk Veteriner Hekimleri Dernegi Dergisi:* Nisan, 1953.

Fasciola gigantica referred to in the literature as occurring in East Africa, Central Asia, the Philippines and the southern half of the U.S.A. appears also to be widespread in Turkey.

In 1941 a number of losses among the Karacabay Merino sheep farms on the shores of Lake Abolyont were found to be caused by this parasite. In the summer and autumn of 1946 a flock of 245 sheep and goats were attacked in the Dortyol district. The disease occurring for four months involved 202 sheep and 8 goats, and the losses amounted to 85 per cent. In 1952, in September, the disease broke out among the sheep and goats of the Kirikhan district. It is very probable that this trouble was responsible for considerable losses in Southern and Western Turkey. The disease does not show the same classical symptoms as the other types of distomatosis. The high mortality and the lung lesions make a differential diagnosis possible and the morphology of *Fasciola gigantica* is sufficient to distinguish it from *Fasciola hepatica.*

In the Kirikhan district it was necessary to make a differential diagnosis between *Fasciola Gigantica* and pasteurellosis and blue tongue.

The Kirikhan outbreak occurred on the shores of Lake Amik and on the banks of the Karasu River. The majority of the land in this region is given over to the production of rice and cotton. In the year 1952 the pastures were dried up by lack of water and the sheep had to graze on grass growing close to the river and the lake. The disease broke out with the deaths of four or five sheep a day and the owners, thinking it was an outbreak of ordinary liver fluke disease, dosed them with carbon tetracchloride without any effect. The outbreak involved something in the region of a thousand sheep.

There are no symptoms that may be accepted as diagnostic; the disease attacks sheep and goats of all ages and both sexes. There was prostration, inappetence, and a rise of temperature to 42°C. The fleece came out when pulled with the fingers but there were no tetanic symptoms. Abortion took place in a number of isolated cases. Usually the animals lived for two to three days and only rarely did they last for five or six days.

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No changes were observed on the skin or subcutaneously post mortem, and although there was emaciation there were no changes in the flesh. There were no petechiæ or anything resembling the lesions of a septicæmia. The abdominal cavity contained two to three litres of a red fluid. Neither the spleen, the kidneys, the intestines nor the thoracic cavity showed any pathological changes.

The liver, however, was covered by a gelatinous yellow capsule which was adherent to the diaphragm. The gall bladder was enlarged and filled with thickened and bloodstained bile.

The liver capsule showed many nodules about the size of a hazel nut and when these were incised they were found to contain coagulated blood and *Fasciola gigantica*. There were a number of parasites in the bile ducts and in the liver substance. On incision the liver substance showed a cirrhosis.

As it is well known, *Fasciola gigantica* very often gives rise to lung lesions and in this case a few nodules, that is to say, one to four in each lung, were observed. These were about the size of a 25 piastre piece and on incision were found to contain coagulated blood and the parasite.

As far as curative measures are concerned, carbon tetrachloride produced no results whatsoever. Good results were, however, obtained by using Avlothane, which is a proprietary brand of Hexachlorethane and it appears to be specific in this disease.

R. H. M.

HISTORICAL NOTE

THE VETERINARY SURGEON IN THE MOGUL EMPIRE

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THE Ain-i-Akbari, the account of the state of the empire of the great Mogul Emperor Akbar was drawn up by Abu il Fazl Allami in 1600 and is a work of the most outstanding interest in that it gives, in minute detail, information on the widest variety of subjects. Book of Common Prayer, Queen's Regulations, Mrs. Beeton's Cookery Book, Stonehenge Rural Sports, Helena Rubinstein's Cosmetics Manual and the Highway Code, it is all these works rolled into one, and if many copies were available shortly after its production it must have been instrumental in raising the intelligence quota of the Akbar's subjects to a very high level. On the one hand we learn of His Majesty's inventions for improving the fire power of his armies, on the other we have a recipe for Kabab, " Take 10 lbs. of meat, ½ gallon of ghi, coriander seed, pepper, cardamums, etc.—sufficient for 7 people. . . . " We are advised that Santuk (1 Civet, 1 Chuwa and two bottles rose water) keeps the skin fresh and that prices of Yazd gold satin brocade were stabilised at from 15-150 mans, and that Kashmiri caps could be got for 1 rupee. We hear that Fancy Bazaars were held so that " for the good, the

dawn of success should rise and the wicked dealers be called to account." We have extracts from the works of the leading poets. We learn of the arrangements in the Imperial Harem where, in spite of the multitudes within the gates, His Majesty is a great friend of good order and propriety in business. We have in addition accounts of pigeon racing, deer and camel fights, hawking, polo and leopard hunting.

On the subject of the Royal Stables the work is of absorbing interest. Five stables were kept, those of the Imperial Elephants, the Imperial Horses, the Camels, the cows and the mules. The Ain-i devotes the greatest care to the consideration of each of these topics: we have first an account of the animals in question, the types of breeds, the signs of health and the marks of a good beast: then a list of the stable personnel, followed by feeding regulations, an account of the harness and finally a section on the " Fines " which were collected from the grooms and stockmen when any animal injured itself, lost condition or died. It is laid down, for example, that a good elephant should be 8 dast high, 9 dast long, and 10 dast round the body, nine members, the four feet, trunk, tusks, penis and tail should all touch the ground—and then follows the curious statement that " the elephant, like man, lives to the age of 120 years." The death of an elephant resulted in a fine of three months' wages being extracted from the grooms and if a female died from want of care or starvation the stable boy had to pay the entire value, often something in the region of 5,000 rupees. The driver who mixed drugs with the food of an elephant to make it " hot" and overdid the dose and brought about its demise was liable to suffer capital punishment, have a hand cut off or be sold as a slave. The cows, besides providing milk and butter, were used as pack and draught animals, the Bengali and Dakhin breeds being considered particularly good, kneeling down to be loaded. Mules were popular and as the Ain-i tells us, " In many countries just princes preferred travelling on them because subjects could more readily approach the Sovereign and lay their grievances before him than if he were mounted on an elephant." The camel's great potential value to the state and its capacity for carrying burdens and existing on comparatively little food and water singled it out for special attention and large numbers were kept in the Emperor's stables with meticulous care; twice a year " people endowed with the jewel of insight inspected them as to their leanness or fatness " and the nostrils were regularly injected every year with a mixture of brimstone and simsim oil to obviate fly strike.

The arrangements in the horse stables are perhaps of the greatest interest for here, in addition to the details given for the other menages, we find the presence of a Veterinary Surgeon, the Baytar, on the list of the personnel, and while we are given no information as to the duties of this official we learn a fact of considerable importance, namely, that he held the rank of an Ahadi. The position of an Ahadi in Akbar's government is best understood from the Ain-i itself which tells us " There are many brave and worthy persons whom His Majesty does not appoint to a Mansab (high official), but whom he frees from being under the orders of any one. Such persons belong to the immediate servants of His Majesty, and are dignified by their independence. They go through the

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school of learning their duties, and have their knowledge tested." . . . "A fit person has also been selected to introduce to His Majesty such as are candidates for Ahadiships. Without partiality or accepting bribes, he takes daily several before His Majesty, who examines them. When they have been approved of, they pass through the Yad-dasht, the Ta liqa, the descriptive roll, and accounts. The paymaster then takes security and introduces the candidate a second time to His Majesty, who generally increases his pay from an eighth to three-fourths, or even to more than six-sevenths." We see from this that even at this early time when Veterinary Surgery in England was still in the hands of Farriers and in the grip of witchcraft that considerable strides had been made in India to establish the standing of the profession and set Veterinary Science on a proper footing.

REVIEW

INDEX OF TREATMENT, by W. Hamilton Kirk, M.R.C.V.S. Third Edition, 1954. London: Baillière, Tindall & Cox. Price 45s. net.

THAT a veterinary textbook should run into three editions within six years of its first appearance is sufficiently unusual to indicate clearly that it satisfies a demand: Kirk's "Index of Treatment" must therefore have established its position among veterinary clinicians and the reviewer need do no more than indicate the new features of this latest edition.

The general layout is unchanged from that adopted previously but numerous alterations and additions have been made so that the text is considerably lengthened, not only in the discussions of entries previously included, but by the introduction of entirely new sections as, for example, the chapter on the antibiotics. Every effort has obviously been made to bring the text up to date and this aim has been achieved so far as is possible in the present rapid development of veterinary therapeutics.

The book remains a very personal production and the author's views will not always command universal assent. This, however, is not only inevitable but is by no means undesirable, being vastly preferable to an indecisive medley of all the possible treatments uncritically presented. Most readers will warmly welcome this new edition, some will criticise, there can be none who can afford to ignore it.

NOTICE

GLAXO LABORATORIES, LTD., announce the introduction, from January 24, of a half-strength Stretopen Uterine Tablet which will be known as "Streptopen '250' Uterine Tablets." These tablets are primarily intended for post-partum infections in ewes and sows.

The existing pack of Streptopen Uterine Tablets will in future be known as "Stretopen '500' Uterine Tablets."